VOLUME XVIII JUNE, 1935 NUMBER 6

# JOURNAL OF DAIRY SCIENCE

PUBLISHED BY THE
AMERICAN DAIRY SCIENCE ASSOCIATION

## Contents

*The effect of mastitis upon milk production.* A. O. SHAW and A. L. BEAM ..... 421

*The hydrogen-ion concentration of creamery waters and their relationship to washing butter.* N. S. GOLDING ..... 433

*The bacteriology of Swiss cheese. II. Bacteriology of the cheese in the press.* W. C. FRAZIER, D. A. BURKEY, A. J. PETERS, O. F. SANDERS, and E. I. MAYKUTH ..... 451

*Influence of season and advancing lactation on butterfat content of Jersey milk.* R. B. BECHDEL and P. T. DIX ARNOLD ..... 461

*Behavior of casein salts in a study of a hydrogen-ion phenomenon in the rennin coagulation of heated milk.* JOSEPH E. POWERS and L. S. PALMER ..... 471

*American Dairy Science Association announcements:*
*Committees of the Manufacturing Section* ..... 476
*Program of Annual Meeting—June 24th to 27th, 1935* ..... 477

THE SCIENCE PRESS PRINTING COMPANY
LIME AND CHRIST STREETS, LANCASTER, PA.
*Made in United States of America*

# THEORY and PRACTICE
## MEET IN THE NEW
# WIZARD COIL VAT

The new CP Wizard Coil Vat incorporates every theoretical advantage—made practical by men who have spent their lives building machines for the dairy industry.

The coils are larger in diameter and of larger tubing. They are placed higher in the vat, which makes for more rapid heat transfer. The geared-head motor drives the coil through a worm gear—the most effective mechanism for the purpose. The coil bearings are placed outside the packing boxes so there is no chance for oil to get into the vat.

But we can't tell you the whole story here. Ask your CP salesman or write the nearest office for complete details about the new Wizard, which has already earned the title "the dairy industry's most useful machine."

*Creamery Package*

THE CREAMERY PACKAGE MFG. COMPANY
1961 West Washington Blvd., Chicago, Illinois

Branches: Boston, Buffalo, Chicago, Dallas, Denver, Kansas City, Los Angeles, Minneapolis, New York, Omaha, Philadelphia, Portland, Ore., Salt Lake City, San Francisco, Seattle, Toledo, Waterloo, Ia.

CREAMERY PACKAGE MFG. CO. OF CANADA, LTD.
447 King St. West, Toronto, Ontario, Canada



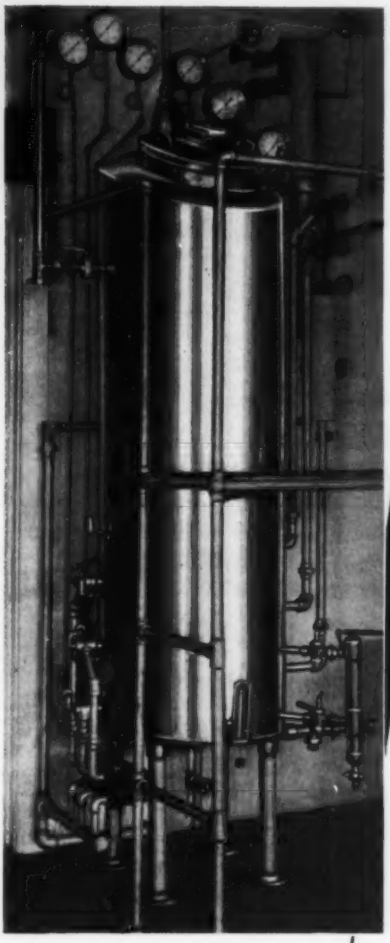
Entered as second-class mail matter April 13, 1923, at the Post Office at Lancaster, Pa., under the Act of March 3, 1879.



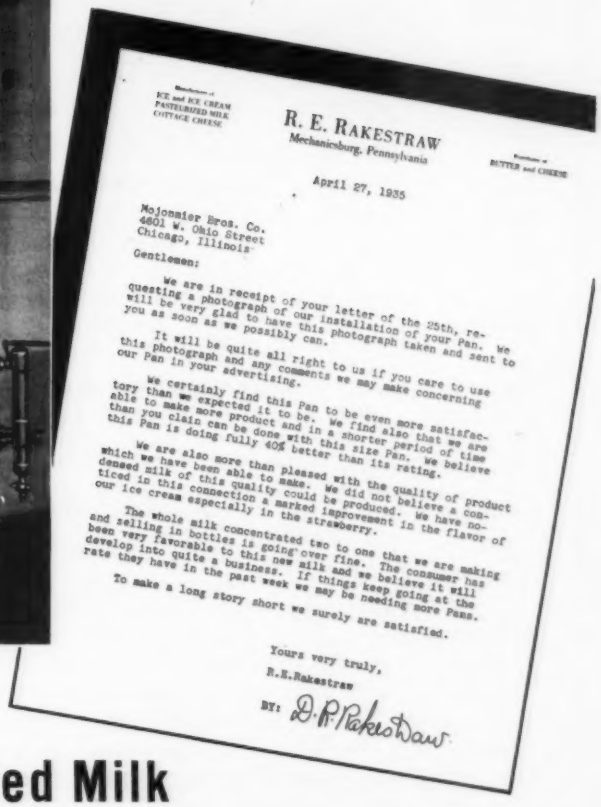
Copyrighted, 1923, by the American Dairy Science Association.

**Note:** The following paragraph should replace the first paragraph on page 305 of the May JOURNAL in which a wrong line appears.

Butterfat free or practically free of lecithin was prepared by the washing process already described. About 500 ml. of the butter oil thus prepared was placed in a liter flask, fitted with a rubber stopper through which were inserted one glass tube reaching nearly to the bottom of the flask and one short tube. The flask then was placed in an oven controlled at 75° C ± 0.5°, and oxygen was bubbled through the butter oil until it became tallowy. The tallowy butter oil then was dispersed in skimmilk of good flavor by means of homogenization, the fat precentage of the remade milk being kept at about 4 per cent.



# A Letter and a Photograph Make a Long Story Short



Manufacturers of ICE and ICE CREAM PASTEURIZED MILK COTTAGE CHEESE

R. E. RAKESTRAW
Mechanicsburg, Pennsylvania

Manufacturers of BUTTER and CHEESE

April 27, 1935

Mojonnier Bros. Co.
4601 W. Ohio Street
Chicago, Illinois

Gentlemen:

We are in receipt of your letter of the 25th, requesting a photograph of our installation of your Pan. We will be very glad to have this photograph taken and sent to you as soon as we possibly can.

It will be quite all right to us if you care to use this photograph and any comments we may make concerning our Pan in your advertising.

We certainly find this Pan to be even more satisfactory than we expected it to be. We find also that we are able to make more product and in a shorter period of time than you claim can be done with this size Pan. We believe this Pan is doing fully 40% better than its rating.

We are also more than pleased with the quality of product which we have been able to make. We did not believe a condensed milk of this quality could be produced. We have noticed in this connection a marked improvement in the flavor of our ice cream especially in the strawberry.

The whole milk concentrated two to one that we are making and selling in bottles is going over fine. The consumer has been very favorable to this new milk and we believe it will develop into quite a business. If things keep going at the rate they have in the past week we may be needing more Pans.

To make a long story short we surely are satisfied.

Yours very truly,

R.E.Rakestraw

BY: D. R. Rakestraw

# FRESH Concentrated Milk

**which is enjoying constantly growing consumer acceptance, can be manufactured successfully only in stainless steel equipment. Mojonnier Stainless Steel Vaccum Pans eliminate any trace of copper contamination and preserve the flavor unimpaired.**

**MOJONNIER BROS. CO., 4601 W. Ohio St., Chicago, Ill.**

**Mojonnier STAINLESS STEEL VACUUM PANS**

**Your advertisement is being read in every State and in 25 Foreign Countries**

# JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

A. C. DAHLBERG, Editor
Geneva, N. Y.

*Associate Editors*

L. S. PALMER, St. Paul, Minn.
C. W. LARSON, Buffalo, N. Y.
H. A. RUEHE, Urbana, Ill.
E. G. HASTINGS, Madison, Wis.
E. B. MEIGS, Beltsville, Md.
PAUL F. SHARP, Ithaca, N. Y.
O. F. HUNZIKER, Chicago, Ill.
L. A. ROGERS, Washington, D. C.
C. E. GRAY, San Francisco, Cal.

*Committee on Journal Management*

O. F. HUNZIKER, Chairman
Chicago, Ill.

R. R. GRAVES, Washington, D. C.
A. A. BORLAND, State College, Pa.

---

*The Journal of Dairy Science* is issued monthly. Subscription is by the volume and one volume is issued per year.

*Manuscripts* should be typewritten and carefully revised before submission to A. C. Dahlberg, New York Agricultural Experiment Station, Geneva, New York. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

*Subscriptions.* Price; $5.00 per volume in United States, Canada and countries within the postal union; $5.50 in countries outside the postal union. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume unless otherwise stated. Renewals should be made promptly to avoid a break in the series.

*Subscriptions and Advertising* should be mailed direct to the Science Press Printing Company, Lime and Green Streets, Lancaster, Pennsylvania.

All subscriptions from the British Isles and British Empire (with the exception of Canada) should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W.C.2, England.

*Correspondence* regarding business policies of the Journal should be addressed to the Editor. The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

VOLUME XVIII JUNE, 1935 NUMBER 6

# THE EFFECT OF MASTITIS UPON MILK PRODUCTION

A. O. SHAW AND A. L. BEAM

*Dairy Department, The Pennsylvania State College, State College, Penna.*

It is generally accepted that each quarter of a cow's udder functions as a separate physiological unit. Parks (1) has given figures for the minimum, the maximum, and the differences in calcium content of milk from each quarter of the udder of five cows. Benton (2), after studying the subject of coagulation, presented values for pH and percentage of butterfat from different quarters of the same udder and concluded that each quarter of a cow's udder is a separate physiological unit. Mattick and Hallett (3) determined the yield, titratable acidity, hydrogen-ion concentration, time of rennet coagulation, total nitrogen, casein nitrogen, amount of butterfat, total solids, and ash content of the milk from different quarters of the same udder. They found wide differences in the results of these determinations in the milk produced by the front and rear quarters, and significant differences when a comparison was made between the secretion from the opposite front, or the opposite rear quarters.

Very little work has been done on the effect of disease on the composition of milk. Cranfield and Ling (4) analyzed milk from a cow which secreted abnormal milk for three lactations and found that the ratio of calcium to phosphorus varied from 0.78 to 1.65. Koestler (5) found that abnormalities in secretion were accompanied by changes in composition which follow a general rule. The amount of globulin, chlorine, sodium and sulphate were increased, while the lactose, potassium, magnesium, calcium, and phosphorus content were decreased.

Mastitis is very common in all parts of the world, and the most conservative estimates place the rate of infection of all cows of producing age at from 15 to 40 per cent. Steelman, according to Rosell (7), in an examination of 170 herds in Germany, found that 52 per cent of all the cows possessed diseased udders, while the workers at the Kiel Station in the same country report that 66 per cent of some 50,000 milk samples examined by them yearly gave evidence of the disease. Minnott (8) reported the examination for streptococci of 850 milking cows in sixteen herds in Great

Received for publication February 1, 1935.

Britain. He found that not less than 333 of these cows were infected. The incidence in each herd ran from 11 to 65 per cent. Hucker (9) reporting in this country found that 90 per cent of 221 cows examined, showed induration or fibrosis of the udder in one or more quarters. Forty-eight per cent of all quarters discharged streptococci in the milk, while 9 per cent showed pus pockets on post mortem.

During the past year a study of the efficiency of the various tests (brom thymol blue, chlorides, cell count, and catalase) for the detection of mastitis has been carried on at The Pennsylvania Station. During the progress of this work, cows were found which gave a positive reaction to the tests in one or more quarters of the udder for a period of time, and then later seemed to be free from any abnormal udder condition as indicated by at least three of the four tests employed. This raised the question as to the effect of such infections upon the efficiency of the secretion from the diseased quarters as compared with the secretion from the opposite healthy quarters.

Some work (6) has been reported which indicates that the rear quarters produce more milk than the fore quarters, and that a variation is often found between the amount of secretion in the fore quarters, and also between the production of the rear quarters.

In order to determine the extent of this variation, eighteen cows with normal udders were selected. For a period of four to six months these individuals gave a negative reaction to the four tests for mastitis. All tests, both for mastitis and butterfat, were run every two weeks. The cows were milked by hand into a bucket divided into halves so that the milking could be carried out in the normal manner. The milk from each quarter was weighed and the percentage of butterfat determined by the Babcock test. The average amount of milk, pounds of butterfat, and percentage of butterfat from each of the four quarters of the udders of these eighteen cows is given in table 1.

TABLE 1

*The average amount of milk produced from each of the four quarters of non-infected udders*

| QUARTER | AVERAGE MILK PRODUCTION | AVERAGE BUTTERFAT PRODUCTION | AVERAGE BUTTERFAT |
|---|---|---|---|
| | *Lbs.* | *Lbs.* | *Per cent* |
| Right Front | 2.9 | .1056 | 3.64 |
| Left Front | 2.6 | .0919 | 3.53 |
| Right Rear | 3.3 | .1178 | 3.57 |
| Left Rear | 3.3 | .1195 | 3.62 |

From these figures it is evident that only a small variation may exist between the amounts of milk secreted by the fore quarters and by the rear

quarters of non-infected udders. The average variation in the pounds of milk produced by opposite quarters ranged from zero for the rear quarters to ten per cent for the fore quarters. The average variation in pounds of butterfat ranged from one per cent between the opposite rear quarters to twelve per cent between the opposite fore quarters.

For the remainder of the study 86 cows were selected which showed an infection in one quarter of the udder as indicated by a positive reaction to at least three of the four tests employed, while the opposite quarter remained negative to all tests.

These quarters were milked by the same method employed on the non-infected udders, and the same data recorded. A considerably greater difference was found to exist between the production of the non-infected quarters and the opposite infected quarters than in the trial where both quarters were normal.

The data in table 2 show that the non-infected fore quarters produced 0.8 of a pound or 34.8 per cent more milk, and 0.0379 of a pound or 34.3

TABLE 2

*Comparison of production from opposite non-infected and infected fore quarters*

| QUARTER | NUMBER | AVERAGE MILK PRODUCTION | AVERAGE BUTTERFAT PRODUCTION | AVERAGE BUTTERFAT |
|---|---|---|---|---|
| | | *Lbs.* | *Lbs.* | *Per cent* |
| Non-infected | 41 | 2.3 | .1102 | 4.79 |
| Infected | 41 | 1.5 | .0723 | 4.82 |

per cent more butterfat than did the opposite infected quarters. If the variation between the milk production of normal quarters as indicated in table 1 is taken into consideration, then there is a difference of approximately 25 per cent more milk produced by non-infected than by infected fore quarters. In the case of butterfat the difference is around 21 per cent in favor of the non-infected quarters.

The non-infected rear quarters as indicated in table 3 produced 1.2 pounds or 31.6 per cent more milk and 0.058 of a pound or 40 per cent more butterfat than the opposite infected rear quarters. There was practi-

TABLE 3

*Comparison of production from opposite non-infected and infected rear quarters*

| QUARTER | NUMBER | AVERAGE MILK PRODUCTION | AVERAGE BUTTERFAT PRODUCTION | AVERAGE BUTTERFAT |
|---|---|---|---|---|
| | | *Lbs.* | *Lbs.* | *Per cent* |
| Non-infected | 45 | 3.8 | .1448 | 3.81 |
| Infected | 45 | 2.6 | .0868 | 3.34 |

cally no difference in the production of milk or butterfat between the non-infected rear quarters, as indicated in table 1, therefore the non-infected rear quarters are approximately 31.6 per cent more efficient in milk production and 40 per cent more efficient in butterfat production than the infected rear quarters.

A comparison of the production of all the opposite non-infected and infected quarters is given in table 4. It will be noted that the non-infected

TABLE 4

| QUARTER | NUMBER | AVERAGE MILK PRODUCTION | AVERAGE BUTTERFAT PRODUCTION | AVERAGE BUTTERFAT |
|---|---|---|---|---|
| | | *Lbs.* | *Lbs.* | *Per cent* |
| Non-infected | 86 | 3.1 | .1280 | 4.13 |
| Infected | 86 | 2.1 | .0798 | 3.80 |

quarters produced on the average one pound of milk and 0.0482 of a pound of butterfat more than the infected quarters. This is equivalent to 32.3 per cent more milk and 37.6 per cent more butterfat from the non-infected quarters than was produced by the infected quarters.

A variation in milk and butterfat production was found to exist between the opposite fore quarters and the opposite rear quarters of non-infected udders. These figures are given in table 1.

After allowing for the maximum variation in milk and butterfat production found between non-infected quarters, it would appear that the non-infected quarters are about 22 per cent more efficient in producing milk and 24 per cent more efficient in butterfat production than the mastitis infected quarters.

## CONCLUSIONS

1. Only a small variation exists between the pounds of milk, per cent butterfat, and pounds of butterfat produced by opposite non-infected quarters of a cow's udder.

2. A considerable variation may be found between the production of opposite mastitis infected and non-infected quarters of a cow's udder.

3. Mastitis infection apparently reduced milk production approximately 22 per cent and butterfat production 24 per cent after allowing for the maximum variation found in the milk and butterfat production of non-infected quarters.

## REFERENCES

(1) PROKS, DOCTEUR J. "De L'Individualite Des Glandes Mammaries Chez La Vache. Le Lait 8, No. 77, p. 553. (Puillet Aaut 1928)

(2) BENTON, ANNE G. "Variations in the Milk from Different Quarters of the Same Udders: Their Significance in Studies of Coagulability." JOURNAL OF DAIRY SCIENCE, 12: 481.

(3) MATTICK, E. C. V., AND HALLETT, H. S. "Variations in Some Chemical and Physical Properties of the Milk from Individual Quarters of a Cow." Jour. Dairy Res. 1: 35.
(4) CRANFIELD, H. T., AND LING, E. R. "Variation in the Composition of the Milk of an Abnormal Cow. Jour. Agr. Sci., 19: 491. July, 1929.
(5) KOESTLER. Mitt. Lebensen. Hyg., 11: 154. 1920.
(6) SWOPE, W. D. Unpublished Data. 1930.
(7) ROSELL. De L'Agriculture De La Province of Quebec. 1933.
(8) Proceedings of the Eeleventh International Veterinary Congress, London. 1930.
(9) HUCKER, G. J., TRUDELL, FERDINAND, AND JENNINGS, W. S. "The Incidence and Detection of Sub-clinical Streptococcus Mastitis." New York Tech. Bul. 199. August, 1932.

# THE HYDROGEN-ION CONCENTRATION OF CREAMERY WATERS AND THEIR RELATIONSHIP TO WASHING BUTTER*

N. S. GOLDING

*University of Idaho Agricultural Experiment Station, Moscow*

## INTRODUCTION

The washing of butter in the grain, after churning and before working, has been an accepted practice for many years; it being considered good practice to wash the butter twice, in such a manner that the last wash water leaves the churn free from buttermilk. However, excessive washing of butter tends to produce a flat flavor (5), but is recommended for poor quality butter.

The biological purity of the water used for washing has received considerable attention (4, 9, 13). Poor keeping quality and actual defects in the flavor of the butter at times have been attributed to infection of butter with undesirable organisms from the wash water (13, 14).

The mineral constituents of the creamery water have received their chief attention insofar as they affect the boiler and the creamery equipment that has to be washed (2). On the other hand, the reaction of the water used for washing butter has not received much consideration.

Knowing that several creamery supplies are alkaline in reaction, about pH 7.6, it was thought that the texture of butter under certain conditions might be affected by washing with such water. It is known that several proteins, *e.g.*, casein, are more soluble in an alkaline reaction than an acid reaction; therefore, were a protective layer removed from the grains of butter by washing with alkaline water more free fat would be liberated (19). Such a theory might possibly answer some of the unknown causes of short grained and sticky butter (19). On the other hand, certain proteins, such as casein, form salts with alkalies and alkaline earths under alkaline conditions, which might be adsorbed on the grains of butter and possibly cause texture defects. Where the texture of butter is found to be adversely affected by alkali wash waters, it would be a relatively simple matter to standardize the water to a most desirable pH, using hydrochloric or some other harmless acid.

Therefore, it is the object of this work to determine the prevalence of alkaline water in Eastern Washington and Idaho and what effect the reaction of such water has on butter which is washed with it.

* Published with the approval of the Director as Research Paper No. 130 of the Idaho Agricultural Experiment Station.

Received for publication February 12, 1935.

## LITERATURE

The early work of this station by C. W. McCurdy (7, 8) shows that water in Idaho, particularly in the dry belts and the irrigated areas, is likely to be alkaline in reaction.

Wright (17), in an extensive study of surface and subsurface waters of the Yakima and Klamath reclamation projects, gives the pH of the water from 79 domestic wells, which appears in table 1, and shows that all these waters are more or less alkaline in reaction, about one-quarter being over pH 8.0.

TABLE 1

*The reaction of waters from domestic wells in the Yakima and Klamath project (17)*

| NUMBER OF WELLS | | |
|---|---|---|
| Ranging from pH 7.0 to 7.4 | Ranging from pH 7.5 to 7.9 | Ranging from pH 8.0 and above |
| 46 | 15 | 18 |

Larsen *et al.* (6), washing butter with alkaline wash water, found that unsalted butter deteriorated one point in flavor more than the control. With salted butter no difference in flavor was noted. Their work does not exclude deterioration that was of biological origin.

Brown (1) conducted experiments on the curd content of butter and showed that the larger the grains of butter in the buttermilk before washing the greater was the curd content of the resulting butter. Whether the removal of the curd was entirely physical or partly chemical was not shown.

Several authorities (12, 15) show that the substance adhering to or adsorbed on the fat globules is of a protein-like nature. The examination of the nature of the protein surrounding the fat globules in milk by Titus et al. (15) lead them to conclude that it is very closely related to, if not identical with, casein. On the other hand, Palmer and Wiese (12) find that the material, most closely adsorbed on the surface of the fat globules in cows' milk, is composed of a mixture of protein and phospholipides. This protein's physical properties, percentage of nitrogen, sulfur, and phosphorus do not correspond with those of any other milk protein. It constitutes the major part of the so-called fat globule membrane. It is unnecessary to go further into the discussion of this still debatable question but rather to accept the general conclusion, that they and several other investigators find, that the substance surrounding the fat globules is largely of a protein-like nature. Since milk when treated with ether does not give up its fat, or only to a slight extent, until a small amount of acid or alkali

has been added to dissolve this protein (15) it would seem that the reaction of the wash water might be of significance in the manufacture of butter.

## MATERIALS AND METHODS

*Natural Waters:* With the exception of the pH determination made on the various creamery water supplies, the water used in these experiments was the supply of the Washington State College at Pullman, which at all times during the investigation showed a pH of 7.6. Its reported analysis by the Cochrane Corporation, Philadelphia, Pennsylvania, is given in table 2.

TABLE 2

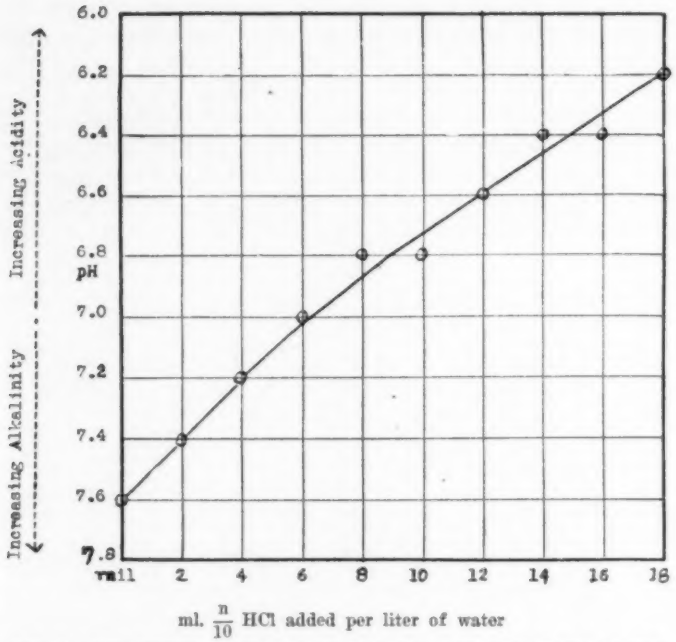
*Analysis of water supply at Washington State College*

| | PARTS PER MILLION | EQUIVALENT PER MILLION |
|---|---|---|
| Calcium (Ca) | 24.2 | 1.21 |
| Magnesium (Mg) | 12.4 | 1.01 |
| Sodium (Na) | 30.8 | 1.34 |
| Iron oxide of alumina ($R_2O_3$) | 4.0 | 0.22 |
| Silica ($SiO_2$) | 48.0 | 1.59 |
| Bicarbonate ($HCO_3$) | 207.0 | 3.39 |
| Carbonate ($CO_3$) | | |
| Sulphate ($SO_4$) | 8.0 | .17 |
| Chloride ($Cl_2$) | Trace | |
| Volatile & organic | 10.0 | |
| Total solids in solution | 240.0 | |
| Suspended matter | Trace | |
| $CO_2$ Free | 14.4 | 0.65 |
| Hardness in terms of $CaCO_3$ | 111.0 | 2.22 |
| Free Acid, pH | 7.8 | |

*Hypothetical combinations*

| | GRS./GAL. |
|---|---|
| $CaCO_3$ | 3.56 |
| $MgCO_3$ | 2.51 |
| $SiO_2$ | 2.80 |
| Iron and oxide of alumina ($R_2O_3$) | 0.23 |
| Sodium carbonate ($Na_2CO_3$) | 3.62 |
| $Na_2SO_4$ | 0.70 |
| NaCl | Trace |
| Volatile of organic | 0.58 |
| Total solids in solution | 14.00 |
| Suspended matter | Trace |
| $CO_2$ | 0.82 |

The buffer curve for the Washington State College water supply is given in figure I, which shows that considerable acid must be added to the water before pH 6.6 is reached, which is about the reaction of sweet cream.




ml. $\frac{n}{10}$ HCl added per liter of water

FIG. I. THE BUFFER VALUE OF THE NATURAL ALKALINE WATER SUPPLY. TEMP. 59° F.

*Distilled Water:* The distilled water when used for washing the butter was double distilled but showed a pH of about 6.6 due to dissolved $CO_2$. Both waters were stored in Pyrex glass. Neither water contained any measurable quantity of nitrogen when determined by the Kjeldahl method. In spite of this, separate duplicate blank determinations were run with each experiment.

*Acids:* Where required the natural water was acidified with C.P. lactic acid in the commercial trials and redistilled N/10 hydrochloric acid when washing the small lots of butter.

*Cream:* The sweet cream was obtained from the University of Idaho herd of Jersey and Holstein cows and was pasteurized before separation.

The commercial cream was sour gathered cream, from two local creameries, neutralized and pasteurized in coil vats in the creamery.

*pH Measurements of Water:* All pH measurements of water were done by colorimetric methods against known buffer standards and checked with several indicators, which were prepared by the method given in the Handbook of Chemistry and Physics (3). A further precaution was taken to

standardize the indicators electrometrically to the pH of the mid-point of their color change. The determinations were made at about the temperature of washing butter as it was considered this gave a better index of the conditions to which the butter was subjected during washing.

*pH Measurements of Cream and Butter:* The quinhydrone method, using a gold electrode and a Leeds Northrup No. 7654 pH indicator, was used to determine the pH of the cream and butter serum. The readings were made at 25° C. The butter serum was prepared by centrifuging according to the method of Weckel (16).

*Method of Washing the Butter:* The commercial churnings, made in a small single roller Cherry churn, were washed with the Washington State College water, tempered and acidified with lactic acid as required. Four buckets of water were used in place of spraying the butter and then a first and second wash water in the usual commercial proportion of about one of butter to two of wash water.

The churnings, to determine the solubility of protein in the various wash waters, were done in a 12-gallon Superior Sanitary crock churn. The 300 grams of butter required in each case were carefully weighed into a 2-litre beaker which contained 600 ml. of water at the required pH. Each series was uniformly stirred, held for 2 hours in a cool room, and the wash water then filtered off from the butter through 4 thicknesses of cheese-cloth, previously washed in distilled water and dried. The wash water was then analyzed for nitrogen according to the following method: (Note: Though a two-hour washing period for butter is not commercial practice, Whittier (18) has shown that equilibrium is attained in this time with milk.)

*Nitrogen determination:* The nitrogen (protein) was determined in the wash water by the Kjeldahl-Gunning-Arnold method (11), a suitable known volume of wash water being used according to whether or not the butter had been previously washed with distilled water. After subtracting the blank determinations the results are expressed in milligrams of nitrogen per litre of wash water. As an additional precaution, ammonia was driven off from the wash water in the Kjeldahl flask before the sulphuric acid was added.

## EXPERIMENTAL

*The Reaction of Creamery Water Supplies:* Through the cooperation of R. L. Nelson and Mr. D. L. Fourt, the pH of a number of creamery waters were determined in the creameries where the butter was washed. The results of these determinations are given in table 3 which shows:

1. A similar distribution to that in table 1.
2. All the waters were more alkaline than the buttermilk made from sweet cream.

TABLE 3

*The reaction of waters from creamery supplies Sept., 1933, to March, 1934*

| LOCATION | NUMBER OF SUPPLIES | | | |
|---|---|---|---|---|
| | Ranging from pH 6.8 to 6.9 | Ranging from pH 7.0 to 7.4 | Ranging from pH 7.5 to 7.9 | Ranging from pH 8.0 and above |
| South East Washington (Determination by R. L. Nelson) | 5 | 5 | 6 | 1 |
| South Idaho (Determination by D. L. Fourt) | | 4 | 4 | |
| Eastern Washington and Western Idaho | 1 | 1 | 3 | |

3. The difference may be considered significant in about half the cases which are pH 7.5 or above.

*The Change in the Reaction of Creamery Water When Used for Washing Butter:* Table 4 gives the results from a set of commercial churnings, churned to grains a little bigger than wheat and washed according to the method described. Table 4 shows:

1. That the natural alkaline waters at pH 7.6 (churnings 1, 3, 6, and 9) were affected in reaction by the first washing of the butter. However, in the second washing the water flowed from the butter practically at the same reaction as when added. Note: The City of Spokane water gave similar results.

2. The waters neutralized to pH 7.0 by acid (churnings 4, 7, and 10) were rendered acid by the first washing of the butter but were unchanged in the second washing.

3. The water adjusted to the reaction of the cream (churnings 2, 5, 8, and 11) remained at practically the same reaction throughout washing.

4. The pH of the serum of the butter, though slightly less acid, when washed with alkaline water was not significantly changed. The greatest difference was between churnings one and two, 0.15 pH.

5. In general it would appear that the surfaces of the grains of butter were affected by the wash water rather than the mass, as the pH of the serum of the butter was unchanged.

*The Change in the Reaction of Wash Water When Used for Washing Butter:* A further series of experiments were conducted on the change in the reaction of wash water, using (a) the natural alkaline wash water, (b) the same acidified to the pH of the cream and (c) water acidified to about the isoelectric point of casein. The washing of the butter was done in 2-litre beakers as previously described. Instead of washing the same butter twice with the prepared waters, distilled water was used so as to remove

TABLE 4

*Change in the reaction of water after being used for washing butter*

| WATER SUPPLY | DATE | CHURNING NO. | CREAM | | pH OF WATER SUPPLY | * ACIDIFIED TO pH | pH OF WASH WATER WHEN RUN OFF FROM THE CHURN | | SERUM OF FINISHED BUTTER pH | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acidity % | pH | | | 1st washings | 2nd washings | | |
| City of Spokane | | | .23 | | 7.6 | not changed | 6.8 | 7.4 | | Large Dual Churn |
| Washington State College | Dec. 23 1933 | 1 | .23 | 5.95 | 7.6 | not changed | 7.4 | 7.6 | 6.2 | Butter made and washed in the small Cherry Churn |
| | | 2 | .225 | 5.95 | 7.6 | 6.0 | 6.1 | 6.1 | 6.05 | |
| | Dec. 28 1933 | 3 | .215 | 6.35 | 7.6 | not changed | 7.0 | 7.4 | 6.65 | |
| | | 4 | .215 | 6.35 | 7.6 | 7.0 | 6.4 | 7.0 | 6.65 | |
| | | 5 | .215 | 6.35 | 7.6 | 6.35 | 6.4 | 6.35 | 6.60 | |
| | Dec. 30 1933 | 6 | .26 | 6.15 | 7.6 | not changed | 6.6 | 7.5 | 6.25 | |
| | | 7 | .25 | 6.15 | 7.6 | 7.0 | 6.4 | 7.0 | 6.30 | |
| | | 8 | .25 | 6.25 | 7.6 | 6.3 | 6.3 | 6.3 | 6.30 | |
| | Jan. 2 1934 | 9 | .20 | 6.5 | 7.6 | not changed | 7.3 | 7.6 | 6.90 | |
| | | 10 | .20 | 6.5 | 7.6 | 7.0 | 6.9 | 7.0 | 7.0 | |
| | | 11 | .20 | 6.55 | 7.6 | 6.5 | 6.5 | 6.6 | 7.0 | |

* With Lactic Acid.

TABLE 5

*Change in the reaction of water when held in contact with butter in the granular form*

| CREAM | | | WATER | | UNWASHED BUTTER HELD IN CONTACT WITH WATER | | BUTTER PREVIOUSLY WASHED ONCE WITH DISTILLED WATER HELD IN CONTACT WITH WATER | | BUTTER PREVIOUSLY WASHED TWICE WITH DISTILLED WATER HELD IN CONTACT WITH WATER | |
|---|---|---|---|---|---|---|---|---|---|---|
| Type | Acidity % | pH | pH | Acidified by adding n/10 HCl pH | 1 hour pH of water | 2 hours pH of water | 1 hour pH of water | 2 hours pH of water | 1 hour pH of water | 2 hours pH of water |
| Sweet Pasteurized | .125 | 6.65 | 7.6 | not changed | | | | | 7.6 | 7.6 |
| | | | | 6.6 | | | | | 6.7 | 7.0 |
| | | | | 4.7 | | | | | 5.2 | 5.8 |
| " " " | .12 | 6.65 | 7.6 | not changed | | | 7.6 | 7.6 | 7.6 | 7.6 |
| | | | | 6.6 | | | 6.6 | 6.8 | 6.8 | 7.0 |
| | | | | 4.6 | | | 5.0 | 5.3 | 5.2 | 5.2 |
| " " " | .125 | 6.65 | 7.6 | not changed | | | 7.6 | 7.6 | 7.6 | 7.6 |
| | | | | 6.7 | | | 6.7 | 6.8 | 6.7 | 6.9 |
| | | | | 4.7 | | | 5.2 | 5.2 | 5.2 | 5.2 |
| " " " | .125 | 6.65 | 7.6 | not changed | | | 7.6 | 7.6 | 7.6 | 7.6 |
| | | | | 6.7 | | | 6.7 | 6.7 | 6.7 | 6.9 |
| | | | | 4.7 | | | 5.2 | 5.2 | 5.0 | 5.4 |
| Sour Cream Neutralized and Pasteurized | .285 | 6.0 | 7.6 | not changed | 6.4 | 6.4 | 7.3 | 7.2 | 7.4 | 7.4 |
| | | | | 6.0 | 6.1 | 6.0 | | 6.2 | 6.2 | 6.3 |
| | | | | 4.7 | 5.0 | 5.2 | 5.0 | 5.2 | 5.4 | 5.4 |
| | .33 | 6.2 | 7.6 | not changed | 6.8 | 6.8 | 7.5 | 7.5 | 7.5 | 7.6 |
| | | | | 6.2 | 6.2 | 6.2 | 6.4 | 6.4 | 6.4 | 6.5 |
| | | | | 4.7 | 5.8 | 5.8 | 5.2 | 5.3 | 5.0 | 5.0 |
| | .33 | 6.25 | 7.6 | not changed | 6.6 | 6.5 | 7.4 | 7.4 | 7.6 | 7.4 |
| | | | | 6.3 | 6.2 | 6.2 | 6.3 | 6.4 | 6.4 | 6.5 |
| | | | | 4.7 | 5.2 | 5.8 | 5.0 | 5.2 | 5.0 | 5.0 |

mechanically, as far as possible, the buttermilk before the acid or alkaline action of the water affected the butter grains.

Table 5, in addition to confirming the statements one and three, in the previous experiment, shows that:

1. In most cases after the wash water was held in contact with the grain of the butter for one hour, the pH of the water remained practically constant till the end of the second hour. Note: In two cases where the period was continued for twenty-four hours the change was still slight.

2. Being more acid, butter made from sour neutralized pasteurized cream affected the change in reaction of the wash water more than sweet pasteurized cream.

3. The acid wash water at pH 4.7 changed most in reaction towards the alkaline side in the second and third washings; at least due in part to its being only very slightly buffered between pH 4.7 and 5.6.

4. The mechanical washing by distilled water removes the free buttermilk, which was the main cause of the change in reaction of wash water towards the pH of the cream.

*The Removal of Nitrogen (Protein) from Butter in the Grain by Washing in Water at Various Hydrogen-ion Concentrations:* Churnings were made in a 12-gallon crock churn and 300 grams of the butter in the grain weighed out into 2-litre beakers containing 600 ml. of water at the required pH. The 3 beakers, containing butter and water in each section, *i.e.*, unwashed butter, washed once with distilled water, or washed twice with distilled water, were treated as far as possible identically except for the difference of pH of the final wash water. After holding in contact with the butter for 2 hours, the filtered wash water was analyzed for nitrogen. The results, the average of duplicates, are expressed in milligrams of nitrogen per litre.

Before discussing table 6, it is as well to point out that the method used of weighing out 300 grams of butter in the grain, even though fairly firm and well rounded, leaves considerable chance for carrying uneven quantities of buttermilk, or water used in the previous washing, into the next wash water. This point most probably accounts for the uneven results in table 6, which only can be considered as showing certain trends, namely:

1. On an average, with the sweet cream butter in the grain, more nitrogen (protein) was removed in the alkaline wash water, less in that which was the same pH as the cream and still less in the acid water, indicating that the alkaline water removed more protein from the butter. Three individual exceptions occur, two in experiment 2, and one in experiment 3.

TABLE 6

*Removal of nitrogen (protein) from butter in the grain by wash waters at various pHs*

| EXPERIMENT | CREAM | | | UNWASHED BUTTER | | | WASHED ONCE WITH DISTILLED WATER | | | | | | WASHED TWICE WITH DISTILLED WATER | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type | Acidity % | pH | Nitrogen per litre of water after washing mg. | Nitrogen per litre of water after washing mg. | Nitrogen per litre of water after washing mg. | *Water for washing pH | Nitrogen per litre of water after washing mg. | *Water for washing pH | Nitrogen per litre of water after washing mg. | *Water for washing pH | Nitrogen per litre of water after washing mg. | Water for washing pH | Nitrogen per litre of water after washing mg. | *Water for washing pH | Nitrogen per litre of water after washing mg. | *Water for washing pH | Nitrogen per litre of water after washing mg. |
| 1 | Sweet Pasteurized | .125 | 6.65 | | | | | | | | | | 7.6 | 31.8 | 6.6 | 30.3 | 4.7 | 27.3 |
| 2 | " | .120 | 6.65 | | | | 7.6 | 35.0 | 6.6 | 34.7 | 4.6 | 35.3 | 7.6 | 12.6 | 6.6 | 10.1 | 4.6 | 10.4 |
| 3 | " | .125 | 6.65 | | | | 7.6 | 34.3 | 6.7 | 31.5 | 4.7 | 30.8 | 7.6 | 10.1 | 6.7 | 10.5 | 4.7 | 10.1 |
| 4 | " | .125 | 6.65 | | | | 7.6 | 48.2 | 6.7 | 46.9 | 5.2 | 45.7 | 7.6 | 22.3 | 6.7 | 19.2 | 4.7 | 16.8 |
| Average for sweet cream | | .124 | 6.65 | | | | | 39.2 | | 37.7 | | 37.3 | | 19.2 | | 17.5 | | 16.2 |
| 5 | Sour Cream Neutralized and Pasteurized | .285 | 6.0 | 142.9 | 107.2 | 135.9 | 7.6 | 13.2 | 6.0 | 12.1 | 4.7 | 11.1 | 7.6 | 4.8 | 6.0 | 4.1 | 4.7 | 3.6 |
| 6 | " | .29 | 6.3 | | | | | | | | | | 7.6 | 4.5 | 6.3 | 2.7 | 4.7 | 2.1 |
| 7 | " | .33 | 6.2 | 87.8 | 78.4 | 93.3 | 7.6 | 9.5 | 6.2 | 9.5 | 4.7 | 8.1 | 7.6 | 3.9 | 6.2 | 3.5 | 4.7 | 2.5 |
| 8 | " | .33 | 6.25 | 107.4 | 112.3 | | 7.6 | 8.7 | 6.3 | 10.0 | 4.7 | 10.1 | 7.6 | 2.8 | 6.3 | 3.4 | 4.7 | 2.8 |
| Average for Sour Neutralized and Pasteurized Cream | | .31 | 6.2 | 112.7 | 99.3 | | | 10.5 | | 10.5 | | 9.8 | | 4.0 | | 3.4 | | 2.8 |

* Acidified with n/10 HCl.

2. With the sour neutralized cream butter, the removal of nitrogen (protein) by the wash water gave too irregular results to draw conclusions, except where the grains had been twice washed with distilled water. Here the general trend was the same, namely: as the acidity increased, the nitrogen (protein) removed diminished.

3. The lack of uniformity in table 6 calls for further investigations by other methods before it can be said definitely that alkaline wash water removes more nitrogen (protein) from the surface of the butter than acid wash waters.

4. Though not intended as a part of this investigation it was noted that the nitrogen in the second and third waters showed a very marked difference when comparing sweet and neutralized cream; the proportion being about four times as great in the former as the latter. As the treatment had been similar in both cases, the problem might form an interesting study.

## DISCUSSION OF RESULT

The presence of water of an alkaline reaction in Idaho and Washington has previously been reported (7, 8, 17). The results of our tests show that such water is frequently used as a creamery supply for washing butter in the grain. The analysis and buffer value of one such water supply (the Washington State College, Pullman) is given in detail and this supply was used for remainder of the experimental work in washing butter in the grain.

The first experiments to determine what happened to the reaction of the butter and the alkaline wash water showed that the water became more acid due to free buttermilk (*i.e.*, toward the pH of the cream) in the first washing of the butter. While in the second washing the reaction of the water remained almost unchanged.

The reaction of the serum of the resulting butter washed twice was not significantly changed by the reaction of the wash water whether or not the wash water used was the same pH as that of the cream or alkaline. Therefore, whatever the effect of alkaline wash water may be, it is a surface effect.

A preliminary study made to determine the effect on the surface of the grains of butter by the change in reaction of the wash water showed a trend for the alkaline water to remove more nitrogen (protein). This removal was greater in proportion after most of the free buttermilk was removed. Owing to the lack of uniformity in results due to an error in the method which has no means of measuring the free water weighed out with the butter, this trend cannot be made a definite conclusion.

The problem is one which warrants further study as there are many texture defects of butter such as greasy and crumbly butter the causes of which are not answered fully. Wode (19) states, "The greasiness of butter

345 may be attributed to a too large amount of free fat and the crumbliness at a low temperature to an abundant crystallization of the free fat.'' If this statement is accepted as a general principle, the breakdown or removal of surface layers would be expected to have a marked bearing on texture defects by increasing the free fat in the butter.

The recommended practice of adding acid to a churn to prevent butter sticking during churning and working (5, 10) may in some ways be associated with alkaline waters. Further, with the introduction of metal churns the reaction of the wash water may play a more significant part. Also as the zeolite process is being recommended for dairies (2) and becoming more common; therefore, an alkaline soda wash water may require to be acidified before it gives the best results for washing butter.

Finally, if there were an advantage to be obtained by standardizing the pH of the wash water, the process could be simply, accurately, and satisfactorily done in most creameries.

## SUMMARY

The reaction (pH) of a number of creamery water supplies in Idaho and Washington have been made at their respective creameries by colorimetric methods. As a considerable proportion of these waters were found to be alkaline in reaction, one such supply was employed to determine the effect of an alkaline water, when used for washing butter.

The first wash water, after washing the butter, was found to have a reaction between that of the water and cream while the second wash water retained the reaction of the original water. On determining the reaction of the serum of the butter, only slight changes were found; therefore, it was concluded that the changes in the reaction of the second wash water were largely a surface effect on the butter.

The nitrogen (protein) removed from butter in the grain by wash waters at various reactions was determined and found to be slightly greater in the alkaline wash waters as compared with those which had been acidified. After extraneous buttermilk was removed, which affected the reaction of the first wash water, the evidence of dissolved protein in the alkaline wash water was more marked.

The effect of alkaline water on the texture of the butter by possibly liberating more free fat in the butter was anticipated, but further experiments are necessary to establish the theory.

## ACKNOWLEDGEMENTS

Thanks are tendered to Mr. R. L. Nelson, State Dairy Inspector, Kennewick, Washington, and Mr. D. L. Fourt, Extension Dairyman, Boise, Idaho, for making pH determinations of creamery water supplies. Appreciation is expressed for assistance and facilities provided at the State College of Washington.

## REFERENCES

(1) Brown, A. M. Curd content of butter and its relation to quality. Agr. Gazette of South Wales, **41**: pp. 905–16. 1930.

(2) Corin, M. F., and Hopkins, E. S. Modern water purification methods for the dairy. Agr. Engin., **11**: pp. 19–22. 1930.

(3) Hodgman, Charles D., and Lange, Norbert A. Handbook of chemistry and physics. 14th Edition, p. 544, Chemical Rubber Pub. Co., Cleveland, Ohio, U. S. A. 1929.

(4) Hood, E. G., and White, A. H. Surface taint butter. Dominion of Canada Dept. of Agr., Pamphlet No. 91, New Series, Ottawa. 1928.

(5) Hunziker, Otto F. The butter industry. 2nd Edition, The Author, La Grange, Illinois. 1927.

(6) Larsen, C., White, Wm., and Bailey, D. E. Effects of alkali water on dairy products. South Dakota Agr. Expt. Sta. Bul. 132. 1912.

(7) McCurdy, C. W. Application of chemistry to agricultural development of Idaho. Idaho Agr. Expt. Sta. Bul. 3. 1893.

(8) McCurdy, C. W. Water and water analysis. Idaho Agr. Expt. Sta. Bul. 8. 1894.

(9) McKay, G. L., and Larsen, C. The keeping quality of butter. Iowa Agr. Expt. Sta. Bul. 71. 1903.

(10) McKay, G. L., and Larsen, C. Principles and practice of butter making. 3rd. Edition, John Wiley & Sons, Inc., New York. 1922.

(11) Methods of Analysis. Association of Official Agricultural Chemists. 18–21, Washington, D. C. 1931.

(12) Palmer, L. S., and Wiese, Hilda F. Substances absorbed on the fat globules in cream and their relation to churning. II. The isolation and identification of adsorbed substances. Journal of Dairy Science, **16**: pp. 41–57. 1933.

(13) Sadler, Wilfred, and Vollum, R. L. The relation of bacteria to the quality of graded butter. Report No. 16, National Research Council of Canada, Ottawa. 1926.

(14) Thomas, S. B., and Morgan, G. T. Some factors influencing the keeping quality of butter. The Welsh Jr. of Agr., **7**: pp. 290–298, Cardiff, Wales. 1931.

(15) Titus, R. W., Sommer, H. H., and Hart, E. B. The nature of the protein surrounding the fat globules in milk. Jr. Biol. Chem., **76**: pp. 237–250. 1928.

(16) Weckel, K. G. An improved method for preparing the serum of butter for pH determination. Journal of Dairy Science, **16**: pp. 249–251. 1933.

(17) Wright, C. E. Surface and sub-surface waters of the Yakima and Klamath reclamation projects. Wash. Agr. Expt. Sta. Bul., 228. 1928.

(18) Whittier, E. O. Buffer intensities of milk and milk constituents. Jr. Biol. Chem., **83**: pp. 79–88. 1929.

(19) Wode, G. Investigations as to the hardness of butter. 10th Worlds' Dairy Congress, Section II, pp. 413–422.

# THE BACTERIOLOGY OF SWISS CHEESE. II. BACTERIOLOGY OF THE CHEESE IN THE PRESS

W. C. FRAZIER, L. A. BURKEY, A. J. BOYER, G. P. SANDERS, AND K. J. MATHESON

*Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.*

The growth and activity of bacteria in the kettle contents during manufacture of Swiss cheese have been discussed in a previous paper (5). It has been shown that of the starter bacteria added to the milk in the kettle, only *Streptococcus thermophilus* grows to any extent in the kettle contents and that it causes little or no change in the acidity of the curd and whey. This paper will deal with studies on the cheese from the time the curd is dipped into the hoop until the cheese has been in the press for 21 hours.

Other workers have noted the presence of streptococci as well as lactobacilli in the cheese in the press. Orla-Jensen (7) observed that streptococci were active during the first hours the cheese was in the press and that the lactobacilli became active later. Thöni (9) found that cocci usually predominate in one day old cheese, but after a short time decrease rapidly, while the lactic rods increase and then make up 80 to 100 per cent of the flora; in small cheeses the lactic rods were found to predominate, even in the fresh mass. Most reported work on bacteria present in cheese deals with later stages of ripening. The growth of *S. thermophilus* and *L. helveticus* Bergey (1934) or *L. casei* Bergey (1923) in the cheese on the press has been referred to in an earlier publication (4) and the resulting development of acidity has been demonstrated.

## METHODS

Samples of the curd were taken by means of a No. 8 cheese trier and only that part of the plug four or more inches from the rind of the cheese was used for either chemical or bacteriological examination except in special experiments. Tests had shown that it was necessary to take samples this far from the rind because the outside of the cheese cools more rapidly, develops acidity at a greater rate, and has a much higher bacterial count than the inside. The one gram sample of cheese curd for bacteriological examination was carefully weighed and was ground with sodium citrate by the method described by Burkey (1): the cheese was placed in a 2¾ inch mortar which had been previously steamed and then cooled to about 50° C. To the cheese in the mortar a few cubic centimeters of water at 50° C. were added from a sterile 9½ cc. water blank. The cheese and water were ground together for one minute; 0.2 gram of sodium citrate was added;

Received for publication February 23, 1935.

the mixture was ground until a smooth homogeneous paste was formed; and finally the remainder of the water of the 9½ cc. blank was added. One cubic centimeter of this mixture contained one-tenth of a gram of cheese and was used in making further dilutions for microscopical and cultural examinations. When slides were to be made, dilutions were made in alkaline 50% alcohol and the preparations were fixed and stained by Burke's modification of the Gram method, as described in an earlier paper (5). For plate counts of bacteria, tomato milk-powder agar was used; citric acid agar was used for counts of aerogenes bacteria, and deep cultures in yeast extract-peptone-sodium lactate agar were used to determine numbers of lactate-fermenting bacteria.

Both types of starter bacteria, the streptococci and the lactobacilli, form chains when they increase in numbers. Consequently, an increase in numbers would be detected by the microscopic method, by which individual cells can be counted, better than by the plate method, which is based on counts of chains or clumps of bacteria. It is true that living and dead cells cannot be clearly distinguished by the usual microscopic method. The counts, however, were made from preparations stained by the Gram method and only the Gram-positive cells were counted. Cells old enough to be Gram-negative were considered dead. Any marked increase in numbers as determined by this method must be considered significant, while a small decrease may mean nothing more than the aging of some of the cells. A comparison of counts by the plate method and the direct microscopic method, is shown in Table 3.

In Table 1 a few counts are given of numbers of living bacteria determined by the method of Frazier and Boyer (3). Most of the counts reported in this paper had been made before this method was developed. The counts in Table 1 permit, however, a comparison of results by the new and by the usual Gram method and given an idea of how near counts of Gram positive organisms are to counts of living organisms.

## RESULTS

Most of the data here reported were obtained from small experimental cheeses of about 50 to 55 pounds, green weight, made at Washington, D. C. Results with full-sized cheeses made at the Grove City Creamery, Grove City, Pa., will be included in the following paper.

A number of studies were made in which samples were taken every hour or every two hours from the time of dipping through the eighth or tenth hour and again at the twenty-first hour. Results indicated that samples at 0, 3, 8, and 21 hours gave a good indication of the growth of the starter bacteria in the cheese; therefore from most cheese only three or four samples were taken. Several workers have shown that bacteria grow

in colonies in the cheese curd. Hence the bacterial count on a gram of cheese may vary somewhat, depending upon the number and size of the colonies in the sample. For this reason the average of results from a number of cheeses is used in Figures 1 and 2. Examples of counts on individual cheeses are shown in Tables 1, 2, and 3, and it will be noted that occasionally a count does not fit well into the general trend of bacterial growth.

TABLE 1

*Numbers of L. helveticus (39a) and S. thermophilus ($C_3$) per gram in a cheese in the press*

| TIME | pH | NOS. OF ORGANISMS—GRAM STAIN | | NOS. OF LIVING ORGANISMS | |
|---|---|---|---|---|---|
| | | 39a | $C_3$ | 39a | $C_3$ |
| HRS. | | | | | |
| 0 | 6.40 | 3,000,000 | 48,000,000 | 970,000 | 35,100,000 |
| 1 | 6.28 | 3,480,000 | 36,600,000 | 1,850,000 | 15,000,000 |
| 3 | 5.94 | 1,500,000 | 77,100,000 | 2,660,000 | 64,000,000 |
| 7 | 5.35 | 630,000 | 231,000,000 | 2,500,000 | 190,000,000 |
| 9 | 5.35 | 630,000 | 248,000,000 | 2,080,000 | 184,000,000 |
| 11 | 5.26 | 11,000,000 | 248,000,000 | 11,600,000 | 187,000,000 |
| 13 | 5.22 | 182,000,000 | 257,000,000 | 184,000,000 | 181,000,000 |
| 22 | 5.06 | 193,000,000 | 349,000,000 | 116,000,000 | 226,000,000 |

TABLE 2

*Numbers of L. bulgaricus (Ga) and S. thermophilus ($C_3$) per gram in a cheese in the press*

| TIME | TEMP. OF CHEESE | pH | NUMBERS OF ORGANISMS | |
|---|---|---|---|---|
| | | | Ga | $C_3$ |
| HRS. | °C. | | | |
| 0 | 50.7 | 6.41 | 1,360,000 | 116,000,000 |
| 1 | 50.1 | 6.24 | 1,360,000 | 101,000,000 |
| 3 | 47.8 | 5.84 | 198,000 | 105,000,000 |
| 5 | 44.5 | 5.79 | 1,180,000 | 83,300,000 |
| 7 | 42.1 | 5.59 | 161,000,000 | 142,000,000 |
| 9 | 39.8 | 5.38 | 787,000,000 | 246,000,000 |

## STARTER BACTERIA

Figure 1 shows the development of acidity as indicated by changes in pH and the growth of the two starter bacteria, *S. thermophilus*, $C_3$ strain, and *L. helveticus* Bergey, 1934, (*L. casei* Bergey, 1923) 39a strain. The data used in plotting the curves up to 9 hours were the average figures from a number of experiments.

Usually *S. thermophilus* decreased slightly during the first two or three hours that the cheese was in the press and then increased at a fairly rapid rate until about the seventh hour. After this the streptococci in-

TABLE 3

*Numbers of starter bacteria and of Aerobacter aerogenes per gram in a cheese and a comparison of counts by the plate and direct microscopic methods*

| TIME | pH | DIRECT MICROSCOPIC COUNTS | | PLATE COUNTS | | |
|---|---|---|---|---|---|---|
| | | 39a | $C_3$ | Gas orgs.[1] | 39a[2] | $C_3$[2] |
| Hrs. | | | | | | |
| Before dip | ..... | 1,350,000 | 15,800,000 | 36,500,000 | 650,000 | 4,700,000 |
| 3 | 5.88 | ..... | 69,300,000 | 7,000 | < 10,000 | 13,300,000 |
| 5 | 5.64 | ..... | 77,000,000 | < 1,000 | < 10,000 | 17,500,000 |
| 9 | 5.40 | 4,720,000 | 292,000,000 | ..... | 200,000 | 117,000,000 |
| 11 | 5.37 | 28,000,000 | 323,000,000 | ..... | 7,300,000 | 97,000,000 |
| 21 | 5.10 | 161,000,000 | 153,000,000 | ..... | 213,000,000 | 31,000,000 |

[1] Counts on citrate agar.

[2] Counts on tomato milk-powder agar.

creased or decreased in numbers slowly and were sometimes slightly higher and sometimes considerably lower in numbers by the time the cheese was 21 hours old (Figure 1). In some experiments the numbers of streptococci were constant for the first three or four hours and in some increased slowly during that time. In most cases there was a rapid increase in numbers thereafter until the sixth to eighth hour. In some cheeses there was a decrease in the numbers of *S. thermophilus* after this first maximum, followed by an increase to a second and greater maximum. This often occurred when a weak lactobacillus culture had been used. In other cheeses

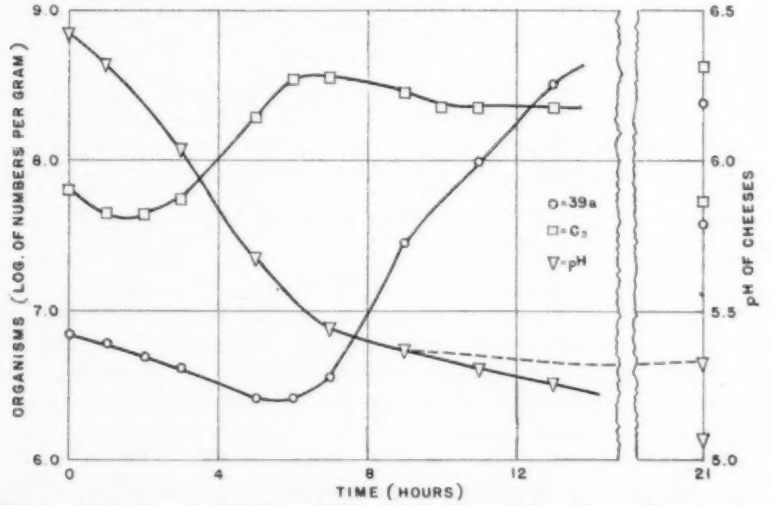



FIG. 1. GROWTH OF *L. helveticus* (39a) AND *S. thermophilus* ($C_3$) IN SWISS CHEESE IN THE PRESS. AVERAGE RESULTS FROM A NUMBER OF CHEESES.

the numbers of *S. thermophilus* decreased gradually and there were comparatively few in the cheese at 21 hours from dipping time. This usually happened when an exceptionally active lactobacillus culture had been used.

As indicated in Figure 1, *L. helveticus* (39a) usually decreased slowly in numbers until about the sixth or seventh hour in the press, after which a fairly rapid increase took place. Individual cheeses often showed considerable variations from the results indicated in Figure 1. The variation which may occur in the curve of the development of the starter cultures and the pH is indicated in Figures 1 and 2 by duplicate symbols. Although 39a started to increase in numbers at about the sixth to eighth hour in the press when a strong starter culture was used, it usually did not start until several hours later when a weak culture was used. In the experiment shown in Table 1, 39a did not start to grow until about the tenth hour and in other cases growth did not start until the eleventh or thirteenth hour. The number of 39a organisms after 21 hours varied considerably in different cheeses. In some cheeses the lactobacilli apparently did not get well started and were low in numbers after 21 hours, while in other cheeses large numbers of 39a organisms were present.

Average figures show a fairly rapid drop in pH of the cheese during the first 7 hours in the press and a more gradual decrease thereafter. Most of this rapid decrease in pH takes place while only *S. thermophilus* ($C_3$) is present in considerable numbers and is growing. Yet during the first three hours in the press the average number of $C_3$ organisms present is only from 43,600,000 to 63,400,000 per gram. This number of organisms seems inadequate to cause the rapid drop in pH which is taking place. The increased size of these organisms, as measured under the microscope, may be an explanation of this unusual activity. Most of the cocci are three to four times as great in diameter and therefore 27 to 64 times as great in volume as cells in a culture grown at ordinary temperatures. In experiments with *E. coli*, Walker and Winslow (10) found an enormous increase in the rate of metabolic activity per cell toward the end of the initial lag period. The activity at this time was greater than that during the period of stable population and that during the phase of logarithmic increase. Later work (11) indicated that the increase in activity per cell could be accounted for partly, but not entirely, by the increase in the size of the cells late in the lag period, and the decrease in metabolic activity per cell during the logarithmic growth phase could be partially explained by a gradual decrease in cell size. Hansen (6) found that the fermentative ability of a thermophilic lactic organism at 55° was 30 times greater than that of *S. lactis* at 20° C. A further explanation of the above effect may be found in the fact that in milk the buffer index is much less when the milk is fresh, at pH about 6.6, than when the acidity has increased to approximately pH 5.2. Consequently, it may be expected that in the

first 3 hours in the press, small amounts of acid can produce relatively large changes in concentration of hydrogen ions.

The pH of the cheese after 21 hours serves as an indication of the activity of the lactobacilli in the cheese in the press. In large cheeses when the lactobacilli have been active, the pH at 21 hours will be about 5.0 to 5.1; if these bacteria were ineffective the pH at 21 hours may be 5.2 or over. Likewise the pH of the cheese after 3 hours is an indication of the activity of *S. thermophilus* ($C_3$) in the cheese. A pH value of 6.05 to 6.1 at 3 hours would indicate that the activity of these streptococci was about normal. A pH of 5.6 at 3 hours would indicate great activity of $C_3$ and a pH of 6.25 somewhat less than normal activity. These pH values will depend, of course, on the pH of the kettle milk and of the cheese curd at dipping time and should be considered as relative rather than absolute values.

Results at Washington, D. C., with small cheeses of about 50 pounds, green weight, indicate that the pH of the curd after three hours in the press should be 5.80 or less, and after twenty-one hours 5.15 to 5.30. These pH values would be obtained when *S. thermophilus* had been very active during the first hours that the cheese was in the press and when *L. helveticus* had been comparatively inactive during the first twenty-one hours. The cheeses were made from a mixed Jersey and Holstein milk with a pH of 6.5 to 6.6 and a titratable acidity of 0.17 to 0.18 per cent. Results were different with large cheeses of the size made in the cheese factories. The relationship between pH values of the large cheeses and quality of the cheeses after they were cured will be discussed more fully in a following paper (No. III of this series).

Figure 2 shows the pH curve and the growth curves of *S. thermophilus* and *Lactobacillus bulgaricus* Bergey, 1934 (Ga). The curves were made from data from a number of cheeses. The culture "Ga" is a lactobacillus grown with a mycoderm. This lactobacillus has a higher maximum temperature than 39a and starts to grow earlier in the cheese. It usually begins to increase in numbers about the 5th hour, but when a weak culture is used growth is considerably delayed. The growth of *S. thermophilus* in cheese made with the Ga culture is similar to its growth with 39a, but average results indicate that $C_3$ reaches its maximum later in cheese containing Ga.

Table 2 shows the results from a cheese made with Ga and $C_3$ cultures, with temperatures of the cheese where the samples were taken. It will be noted that Ga did not begin to grow until the temperature was below 44° C. 39a usually does not begin growth until the temperature falls below 43° C.

The temperature in the interior of large Swiss cheese will drop more slowly than in small ones. In a cheese of about 160 pounds, green weight,

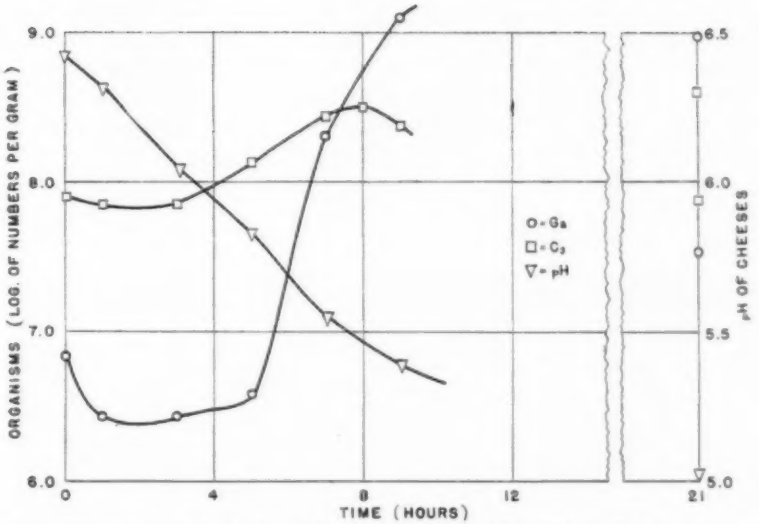



FIG. 2. GROWTH OF *L. bulgaricus* (Ga) AND *S. thermophilus* ($C_3$) IN SWISS CHEESE IN THE PRESS. AVERAGE RESULTS FROM A NUMBER OF CHEESES.

after a cooking temperature of 53.3° C. (128° F.), the temperatures six inches in from the hoop side were 50.5° C. at dipping time, 50° C. after 3 hours, 47° C. after 7 hours, and 39° C. after 21 hours. Both 39a and Ga begin to grow later in these large cheeses than in the smaller (Washington) cheeses. Results of counts on large cheeses at the Grove City Creamery showed that the $C_3$ organisms were in about the same numbers during the early hours in the press as in the smaller Washington cheeses, but that the 39a organisms started to grow about two hours later in the larger cheeses. A common practice in Swiss cheesemaking is to add cool water or whey to the kettle contents just before the curd is dipped out. This reduces the temperature of the curd and makes it more like that of the curd of freshly dipped small cheeses.

## GAS FORMING BACTERIA

As has been reported in a previous paper (5) organisms of the Escherichia-Aerobacter group are able to grow in the kettle contents during the Swiss cheese making process if they are present in large enough numbers in the original milk. That gas forming bacteria of this group are able to grow in the cheese in the press and cause "sounding" or even bloating is a well known fact. Dorner (2) reports that *Bact. aerogenes* is harmful to Swiss cheese and that *Bact. coli* is comparatively harmless. Similar results have been obtained at the Washington laboratories in

TABLE 4

*Effect of added Escherichia communior on cheese with and without added S. thermophilus starter*

| CHEESE NO. | CULTURES ADDED | | | EYES | TEXTURE | GLASS AND CHECKING | FLAVOR | SCORE | GRADE |
|---|---|---|---|---|---|---|---|---|---|
| | L. helveticus | S. thermophilus | E. communior | | | | | | |
| | *Per cent* | *Per cent* | *Per cent* | | | | | | |
| 877 | 1/12 | 1/12 | 1/60 | Too many; round clean | Fair | Trace | Good | 83 | Good No. 1 |
| 877–1 | 1/12 | ..... | 1/60 | Overset, irregular | Fair | None | Sharp | 68.5 | No. 2 |
| 878 | 1/12 | 1/12 | 1/120 | Overset, round, regular | Fair | Checked end | Good | 78 | Fair No. 1 |
| 878–1 | 1/12 | ..... | 1/120 | Nearly nissler | Fair | None | Sharp | 68 | No. 2 |
| 879 | 1/12 | 1/12 | 1/300 | Numerous, small, clean | Fair | None | Good | 79 | Fair No. 1 |
| 879–1 | 1/12 | ..... | 1/300 | Overset, collapsed | Fair | None | Sharp | 68.5 | No. 2 |
| 880 | 1/12 | 1/12 | 1/300 | Round, clean, regular | Fair | Trace at end | Good | 82 | Good No. 1 |
| 880–1 | 1/12 | ..... | 1/300 | Small, irregular, gassy | Fair | None | Biting | 68 | No. 2 |

experiments in which a culture of *Escherichia communior* or of *Aerobacter aerogenes* was added to the kettle milk. Both *S. thermophilus* and *L. helveticus* starters were added to the control cheeses but the streptococcus culture was omitted from the test cheeses. Results of the experiments with *E. communior* are shown in Table 4 in which the description of four typical pairs of cheeses of the series at time of cutting is given. All of the cheeses which received gas organisms but no *S. thermophilus* starter were gassy or bloated in the press. The results showed that *E. communior* did not harm the cheeses when proper starter cultures were used. Figure 3

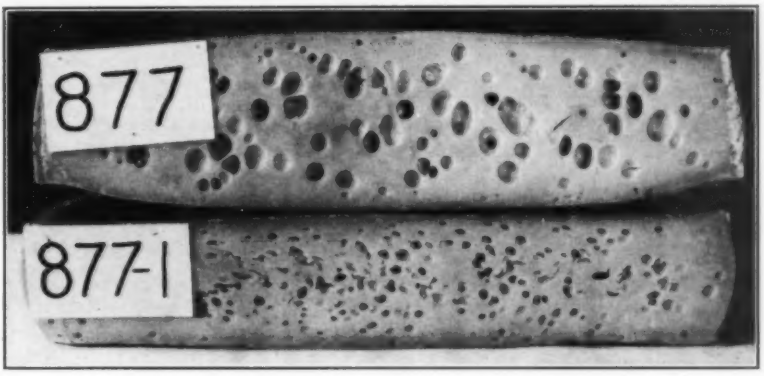



FIG. 3. PAIR OF THREE MONTHS OLD CHEESES MADE AT WASHINGTON, D. C. UPPER CHEESE RECEIVED *S. thermophilus* CULTURE; LOWER RECEIVED NONE. *Escherichia communior* HAD BEEN ADDED TO THE MILK FROM WHICH THE CHEESES WERE MADE.

shows the No. 877 pair of cheeses when cut. In Table 5 are shown the results of similar experiments in which *Aerobacter aerogenes* was used instead of *E. communior*. All cheeses without added *S. thermophilus* were bloated in the press, while cheeses to which this starter had been added showed no signs of gas in the cheese in the press. Most of the cheeses, however, regardless of the starters added were of poor quality. This has been true of all cheeses to which very many *A. aerogenes* organisms have been added, regardless of the starters used and whether or not gas production has been suppressed while the cheese was in the press. In all experiments at these laboratories it has been possible, by the use of good starters, to suppress gas formation which would otherwise result from addition of *A. aerogenes* organisms, but the resulting cheeses have been consistently poor in grade. Figure 4 shows a pair of cheeses cut after 21 hours in the press. Cheese No. 744 received no *S. thermophilus* starter and was bloated in the press. Table 3 shows typical counts of gas and starter organisms in a cheese made from milk to which *A. aerogenes* had

TABLE 5

*Effect of added Aerobacter aerogenes on cheeses with and without added S. thermophilus starter*

| CHEESE NO. | CULTURES ADDED | | | EYES | TURE TEX- | GLASS AND CHECKING | FLAVOR | SCORE | GRADE |
|---|---|---|---|---|---|---|---|---|---|
| | L. helveticus | S. thermophilus | A. aerogenes | | | | | | |
| 885 | 1/12 | 1/12 | 1/1000 | Few, clean, round, fair | Fair | Both | Sour | 73 | Special |
| 885–1 | 1/12 | ..... | 1/1000 | Gassy edge | Fair | None | Sl. rancid | 73 | Special |
| 886 | 1/12 | 1/12 | 1/900 | Few eyes, fair | Good | Many checks | Fair | 68 | No.2 |
| 886–1 | 1/12 | ..... | 1/900 | Gassy edge | Good | None | Sl. rancid | 75 | Poor No. 1 |
| 887 | 1/12 | 1/12 | 1/60 | Few eyes, gassy edge, | Fair | Checks | Sour | 68 | No. 2 |
| 887–1 | 1/12 | ..... | 1/60 | Round, clean | Fair | None | Sharp | 77 | Poor No. 1 |
| 888 | 1/12 | 1/12 | 1/60 | Few eyes | Poor | None | Flat, sour | 67.5 | No. 2 |
| 888–1 | 1/12 | ..... | 1/60 | Few eyes | Poor | None | Flat, sour | 67.5 | No. 2 |

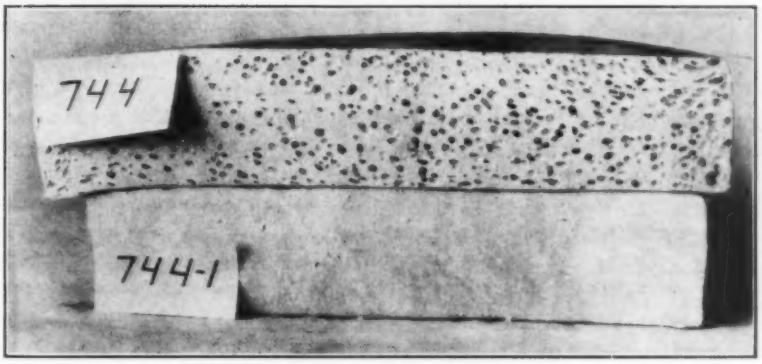



FIG. 4. PAIR OF ONE DAY OLD CHEESES MADE AT WASHINGTON, D. C. THE LOWER CHEESE RECEIVED *S. thermophilus* STARTER; THE UPPER CHEESE RECEIVED NONE. *Aerobacter aerogenes* HAD BEEN ADDED TO THE MILK FROM WHICH THE CHEESES WERE MADE.

been added. The gas bacteria were high in number just before the curd was dipped but decreased rapidly in the cheese and were not found after 3 hours in the press.

It has been our experience in cheese factories that the activity of the lactobacillus starter is also important in restraining the growth of gas-forming bacteria in cheese in the press. At the Grove City Creamery the cheeses usually "sound" or show evidences of gas formation when the cheese has a high pH value after 21 hours in the press, an indication of inactivity of the lactobacillus starter.

The anaerobic gas-forming bacteria of the *Clostridium* group do not usually grow enough in cheese in the press to cause evident changes at that time. Heavy contamination of the milk with spores of these bacteria in combination with weak or inactive starter bacteria may cause gas formation in the press. Usually the action of these anaerobic gas-formers in the cheese takes place later.

### OTHER BACTERIA

Counts on the propionic acid bacteria, *Propionibacterium shermanii*, show that these organisms do not grow in the cheese in the press and in fact may decrease slightly in numbers. Other lactate-fermenting bacteria are able to grow in cheese in the press, however, and under some conditions may be present in large numbers 21 hours after dipping. It was reported in a previous paper (5) that some of these organisms can grow in the kettle contents. Some of them can grow still better in cheese in the press. Counts of tetracocci and of rod-shaped bacteria of the *Lactobacillus casei* Bergey, 1934, (*Bacillus casei* $\alpha$ v. Freudenreich) type in a

large number of cheeses showed that the tetracocci increased from 6,000–70,000, average 10,000 per cubic centimeter, in the kettle milk to 10,000–1,000,000, average 100,000 per gram, after 21 hours in the press. These tetracocci resemble those described by Orla-Jensen (8) and by him given the genus name of *Tetracoccus*. These organisms will be discussed in more detail in a following paper. When these bacteria are added to the kettle milk they sometimes reach large numbers in cheeses after 21 hours in the press. As many as a billion tetracocci or *L. casei* organisms per gram have been found in individual cheeses.

The growth of *S. lactis* is stopped by cooking temperatures in the kettle, as was shown in a previous paper (5). Counts of these organisms in the press show that even if they are present in large numbers when the curd is dipped they decrease rapidly in numbers during the early hours of the cheese in the press and are few in number at the end of 21 hours.

Organisms of the *Lactobacillus lactis* Bergey, 1934, and *Lactobacillus longus* Bergey, 1934, types were seldom found in the cheese in the press and then usually when unsterilized kettle whey had been used as a starter. As many as a billion of these organisms per gram have been found in 21-hour old cheese made with unsterilized whey starter. These cheeses were all of poor grade when cut.

## DISCUSSION

It is evident that both *Streptococcus thermophilus* and a lactobacillus starter are important in cheese in the press. The streptococci are needed to produce an increase in acidity during the first hours in the press. Their action is almost complete after about eight hours and, unless the lactobacillus starts to grow at or before this time, opportunity is given for the growth of undesirable bacteria. An effective lactobacillus culture will continue to increase the acidity and bring the pH value of the cheese down toward 5.0 as a minimum.

Experiments reported in this paper have shown that a good *S. thermophilus* starter will suppress gassiness or bloating of the cheese in the press. Experience has shown that an effective lactobacillus starter is also necessary to keep down the gas-forming bacteria. A culture like Ga which starts to grow earlier than the 39a culture, and which causes a more rapid development of acidity in the cheese would be a more effective starter organism for combatting gas than 39a. Too rapid a production of acid in the cheese in the press, however, is harmful to the quality of the ripened cheese as will be shown in a following paper. Consequently, the addition of large amounts of starter may suppress gas formation in the cheese in the press but may cause other defects in the cheese. As has been stated above, the presence of large numbers of *A. aerogenes* bacteria in the kettle

milk usually foretells a cheese of poor quality, even when the gas formation in cheese in the press has been inhibited. Efforts must be made to keep the numbers of *A. aerogenes* in milk at a minimum if cheese of good quality is to be made.

The maximum acidity attained by the cheese in the first day is of importance in determining the fermentation which will take place subsequently in the cheese. If acid production has been too rapid during the first hours in the press, as indicated by a rapid drop in pH values of the interior of the cheese and too rapid drainage, then the pH at 21 hours will usually be high. The acidity of this cheese will never become as great as it should and, as a consequence, eyes will probably form too early and in too great numbers and the cheese will become overset. If, on the other hand, the early acid production is too slow, which tends to result in slow or insufficient drainage, the acidity of the interior of the cheese at 21 hours is likely to be high; in such a case eye formation will be delayed and the eyes may be too few, too small, or even absent ("blind" cheese). The regulation of the growth and action of the starter bacteria in the cheese in the press affords a means of controlling to a great extent the changes in the cheese both in the press and later in the curing rooms. It must be realized, of course, that proper use of starters will not correct defects due to poor milk, incorrect manufacturing methods, or improper care in the cellars.

## SUMMARY

A study has been made of the bacteriology of Swiss cheese from the time the curd was dipped from the kettle until 21 hours later.

Small cheeses with a green weight of about 55 pounds were used in most of the experiments, but results have been compared to those with large cheeses.

A summary of results showed:

*S. thermophilus* usually started to grow within three or four hours after dipping and increased in numbers rapidly until the sixth or eighth hour. Thereafter the increase in numbers was slow and in some cases the numbers decreased gradually until the twenty-first hour.

*L. helveticus* (39a) usually decreased slowly in numbers until about the sixth to eighth hour in the press and then increased at a fairly rapid rate. The numbers after 21 hours were high with an active starter and low with a weak one.

*L. bulgaricus* (Ga) usually decreased in numbers until about the fifth hour after dipping when a rapid increase in numbers took place.

The pH of the cheese usually dropped more rapidly during the first seven or eight hours than during the later hours. This early drop in pH evidently was due chiefly to the action of the *S. thermophilus* organisms

present. Most of the cells of *S. thermophilus* had a diameter 3 or 4 times and a volume approximately 27 or 64 times as great as that of cells grown at ordinary temperatures. This is one explanation for the large fermentative activity per cell during the first hours of the cheese in the press. Moreover, small amounts of acid can produce relatively large changes in pH during the early hours in the press, since the buffer value is relatively small in the early pH range.

The pH of the cheese at three and at twenty-one hours after dipping may serve as an indication of the effectiveness of the *S. thermophilus* and lactobacillus starters, respectively. The relative change in pH is more significant than the actual pH value.

The action of *S. thermophilus* is about the same in large cheeses as in small, but the growth and action of the lactobacillus cultures usually begin an hour or two later in the larger cheeses, because the interior cools more slowly than in smaller cheeses.

Gas formation and growth by either *Escherichia communior* or *Aerobacter aerogenes* can be suppressed in cheese in the press by use of active starters. If *A. aerogenes* has been present in large numbers in the kettle contents, however, a poor quality cheese will probably result.

*Propionibacterium shermanii* did not grow in cheese in the press. Bacteria of the tetracoccus or *Lactobacillus casei* types usually increased in numbers, if present, and under some conditions attained large numbers by the twenty-first hour.

*C. lactis* died off rapidly in cheese in the press. Organisms of the *Lactobacillus lactis* and *Lactobacillus longus* types were able to grow rapidly but usually were not present in large numbers.

## REFERENCES

(1) Burkey, L. A. Methods for determining the persistence of certain important bacteria in Swiss cheese. (Abstract) Jour. Bact. **21**: 40–41. 1931.

(2) Dorner, W. Zur Frage der Entstehung geblähter Milch. Landw. Jahrb. Schweiz. **39**: 777–792. 1925.

(3) Frazier, W. C., and Boyer, A. J. A method for distinguishing living from dead cells of gram-positive bacteria by stained preparations. (Abstract) Jour. Bact. **27**: 31–32. 1934.

(4) Frazier, W. C., Burkey, L. A., Matheson, K. J., and Watson, P. D. Thermophilic streptococci as starters for Swiss cheese. Journal of Dairy Science **16**: 387–399. 1933.

(5) Frazier, W. C., Sanders, G. P., Boyer, A. J., and Long, H. F. The bacteriology of Swiss cheese. I. Growth and activity of bacteria during manufacturing processes in the Swiss cheese kettle. Jour. Bact. **27**: 539–549. 1934.

(6) Hansen, P. A. Das Wachstum von thermophilen Bakterien. Arch. Microbiol. **4**: 23–35. 1933.

(7) Orla-Jensen, S. Ueber die im Emmentalerkäse stattfindende Milchsäuregärung. Landw. Jahrb. Schweiz. **20**: 287–311. 1906.

(8) ORLA-JENSEN, S. The lactic acid bacteria. Copenhagen. 1919.
(9) THÖNI, J. Beitrag zur Kenntnis der Bakterienflora von nach Emmentalerart bereiteten Käsen in verschiedenen Reifungsstadien. Landw. Jahrb. Schweiz. **23**: 395–421. 1909.
(10) WALKER, H. H., AND WINSLOW, C.-E. A. Metabolic activity of the bacterial cell at various phases of the population cycle. Jour. Bact. **24**: 209–241. 1932.
(11) WALKER, H. H., WINSLOW, C.-E. A., HUNTINGTON, E., AND MOONEY, M. G. The physiological youth of a bacterial culture as evidenced by cell metabolism. Jour. Bact. **27**: 303–324. 1934.

# INFLUENCE OF SEASON AND ADVANCING LACTATION ON BUTTERFAT CONTENT OF JERSEY MILK

R. B. BECKER AND P. T. DIX ARNOLD

*Florida Agricultural Experiment Station, Gainesville*

## INTRODUCTION

Environment and stage of lactation are two factors affecting the fat content of cow's milk. The environmental conditions in Florida—seasonal ranges in temperature, distribution of rainfall and length of grazing season—differ from those of other latitudes. Because of these differences in environment, it was desirable to study the variations in the fat content of cow's milk in this latitude.

## THE STATION HERD AND ITS ENVIRONMENT

The dairy cattle owned by the Florida station are located at Gainesville, in the center of the north end of the Florida peninsula, some sixty miles from the Atlantic Ocean and from the Gulf of Mexico, at an altitude of about 176 feet above sea-level, between 29° and 30° North latitude. The temperatures from 1917 to 1932, inclusive, ranged from an average mean minimum of 46.5° F. in January, to a mean maximum of 90.7° in August. An average annual rainfall of 51.03 inches was reported at Gainesville during these years, of which 27.65 inches fell during June to September, inclusive. The dairy herd always has been limited in numbers of cows. The dairy barn has 36 stanchions, which seldom were all filled. During the earlier years, some high grade Jerseys were in the herd, but were replaced with registered Jerseys in the course of time.

Cows have grazed over the same pasture during this period of years. They were allowed access to pasture daily throughout the year, in the majority of seasons, and were stabled in an open barn only during the milking hours. Silage was fed to supplement pastures, almost to the exclusion of hay, prior to 1929. Since 1929, a very limited amount of alfalfa hay was fed to the higher producing cows. Feeding practices were reasonably uniform otherwise, except with regard to mineral supply, as was pointed out in Florida station technical bulletin 262.

## REVIEW OF LITERATURE

The seasonal influence on butterfat content of milk was observed by Eckles (2) to be independent of feeding practices. "The tendency is for the tests to reach the low point in early summer, usually June, and the highest point in December or January." He (4) stated later that "The

Received for publication February 26, 1935.

effect of the season is apparently the result of weather conditions, especially heat and humidity. . . . during a period of hot, humid weather the percentage of fat is depressed, while if the conditions are . . . dry and cool, the test is increased." With cows fed mainly on alfalfa hay, Headley (8) observed the high average butterfat test in November and the low test in July. He stated that "These results show conclusively that the fat content of milk varies with the season, regardless of the kind of ration used." Baker and Cranfield (1) in a study conducted at milk depots in England, found the highest test in November, and the low average test in June. Hitteher (10) in a study of milk of 16 Holstein cows, noted that in all cases there was a rise in butterfat content of the milk in the month of September, probably due to environment.

Ragsdale and Brody (13) observed 10 cows over 41 days (March 13 to April 22) at the Missouri station. With mean daily temperature ranges between 27 and 70° F., there was a depression of almost 0.2 per cent in the average fat content of the milk, for an increase of 10° F. between the observed temperatures. Hays (7) noted a depression of 0.079 per cent fat per 10 degrees rise in mean temperature over 258 days, from January, 1924, when the entire Missouri station dairy herd was studied under uncontrolled conditions. With two Jersey cows kept in rooms with temperatures maintained more uniformly over periods of about one week duration, the average depression of 0.095 per cent in fat test per 10 degrees rise in temperature, was noted in seven trials between 27 and 92.7° F. In the temperature range between 27 and 72.5 degrees, an average depression of 0.189 per cent fat occurred for each 10 degrees depression in temperature. Above 70° F., an unexpected rise in fat test occurred, perhaps attributable to an increased rate of metabolism with the higher temperature. Temperature, Hays concluded, is a major factor in the seasonal variation in percentage of fat in cow's milk.

Weaver and Matthews (17) analyzed one year's records of dairy cows at the Iowa station, and calculated by application of regression equations that for each degree of increase in atmospheric temperature outside the dairy barn the fat percentage in Ayrshire milk was decreased 0.0017 per cent; in Guernsey milk, 0.0103 per cent; in Holstein milk, 0.0063 per cent; and in Jersey milk, 0.0066 per cent.

It is generally recognized that milk tends to become richer in fat as lactation advances. However, this increase is not continuous from date of calving. Breeds differ as to duration of an interval following parturition, during which a high butterfat test occurs temporarily, this period usually being one-half to three months in length. Eckles and Shaw (4) state that "The per cent of fat on the average, declined during the first three months, followed by a period of 4 or 5 months with but little change. From this point a rapid increase was found to the end of the lactation period."

Hills (9) studied 30 private herds in Vermont, and additional lactation records from herds in Minnesota and New York. He reported that, in general, changes in the fat tests were inverse to changes in temperatures. Also, that the lowest fat tests with two-thirds of the cows, occurred within 2 to 4 months after calving, and the highest tests after the seventh month of lactation. Hogstrom (11) studied 822 lactations, principally by Ayrshire cows, and found that after omitting the colostrum period, the percentage of fat was higher the first month, then decreased until the third month, when it reached the minimum. From there, the butterfat tests increased gradually, attaining the maximum at the close of lactation. Speir (16) reported that the highest fat content of the milk from a group of almost 400 Ayrshire cows was during the first week after calving, and that the lowest average test was during the fourth week, followed by a gradual increase to the end of the lactation. Linfield (12) tabulated the lactations of 16 cows of several breeds in the Utah station herd, and concluded that the percentage of fat decreased slightly in the second month, then gradually increased until the ninth month, after which the rate of increase was more rapid, until the end of the lactation.

Eckles (3) pointed out from a study of 3,154 lactations representing several breeds of dairy cows, that there was a slight decline from the first to second month after calving, followed by a gradual increase to the twelfth month. Moreover, he stated that ". . . the seasonal influence modified the variation in fat content of milk materially, and in fact is a greater force than stage of lactation, except for the rapid increase as the cow is going dry."

## EXPERIMENTAL METHODS AND RESULTS

From the Florida station herd, 293 lactations were obtained by registered Jersey cows. The cows were milked twice daily, and butterfat tests had been made at seven or more monthly intervals during each lactation. The preceding calf had been dropped normally, and no illness or other condition was known which might have an abnormal effect upon lactation.

The 293 lactations were assembled into groups according to the month in which the cows freshened. These groups ranged from 15 cows that calved during May, to 40 during October. The average fat tests were determined by months for each group. All fat determinations had been made by the Babcock method. The number of monthly butterfat tests ranged between 219 in September and October, and 266 during March. These group averages were assembled in such a manner as to show the calendar month of each test, as well as the number of months after calving, giving the group averages equal weighting.

The method of arranging average butterfat tests in 144 cells (twelve rows of cells for calendar months and twelve rows for the successive months

TABLE 1

*Average butterfat percentages in the milk of Jersey cows in the Florida station dairy herd, 1917 to 1932*

| MONTHS AFTER CALVING | AVERAGE BUTTERFAT PERCENTAGES DETERMINED IN THE RESPECTIVE MONTHS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Average |
| First | 4.42 | 4.98 | 4.33 | 5.03 | 4.79 | 4.70 | 4.38 | 4.35 | 4.15 | 4.49 | 4.58 | 5.07 | 4.605 |
| Second | 4.94 | 4.89 | 4.95 | 4.38 | 4.55 | 4.30 | 4.50 | 4.33 | 4.47 | 4.37 | 4.73 | 4.72 | 4.594 |
| Third | 5.05 | 4.90 | 4.74 | 4.70 | 4.17 | 4.65 | 4.58 | 4.43 | 4.58 | 4.70 | 4.76 | 4.95 | 4.684 |
| Fourth | 4.98 | 4.83 | 4.77 | 4.92 | 4.50 | 4.48 | 4.67 | 4.76 | 4.74 | 4.95 | 5.49 | 4.91 | 4.833 |
| Fifth | 4.89 | 5.13 | 4.74 | 5.08 | 4.86 | 4.76 | 4.57 | 4.83 | 5.16 | 5.17 | 5.43 | 5.67 | 5.024 |
| Sixth | 5.41 | 4.89 | 5.04 | 4.79 | 4.56 | 4.90 | 4.76 | 4.58 | 5.08 | 5.01 | 5.37 | 5.50 | 4.990 |
| Seventh | 5.78 | 5.34 | 4.97 | 5.00 | 4.48 | 4.83 | 4.67 | 4.95 | 4.80 | 5.24 | 5.88 | 5.81 | 5.145 |
| Eighth | 5.83 | 5.51 | 5.39 | 4.77 | 5.10 | 4.71 | 5.01 | 5.01 | 5.19 | 5.08 | 5.92 | 6.00 | 5.293 |
| Ninth | 5.77 | 5.63 | 5.51 | 5.45 | 4.76 | 4.94 | 4.82 | 4.88 | 5.29 | 5.41 | 5.50 | 5.51 | 5.289 |
| Tenth | 5.61 | 5.80 | 5.58 | 5.57 | 5.15 | 4.79 | 5.15 | 4.89 | 5.13 | 5.42 | 5.79 | 5.71 | 5.382 |
| Eleventh | 5.58 | 5.93 | 6.01 | 5.39 | 5.07 | 5.43 | 4.88 | 5.13 | 4.99 | 5.76 | 5.79 | 5.99 | 5.496 |
| Twelfth | 5.70 | 5.39 | 5.96 | 5.25 | 5.56 | 5.48 | 5.70 | 4.99 | 5.42 | 5.10 | 6.05 | 6.06 | 5.555 |
| Average | 5.330 | 5.268 | 5.165 | 5.027 | 4.795 | 4.830 | 4.806 | 4.760 | 4.916 | 5.058 | 5.441 | 5.464 | ..... |

after calving) for an analysis of variance (15) gave an even distribution of seasonal influence throughout one arrangement of the data. By this method the row of cells for the first month after calving included data from groups of cows in the first to twelfth months of lactation. When the influence of stage of lactation was spread evenly throughout one set of columns, the purely seasonal influence was measured more exactly by the series of means for each column of data. Likewise, seasonal influence in turn was distributed evenly while the measurements regarding advance in lactation were being made. Such an analysis is shown in table 1, for the average butterfat tests of these 293 lactations.

In general, higher butterfat tests were associated with the cooler months of the year, and the lower tests with the warm summer months. An average of 5.46 per cent butterfat in December contrasts with a low of 4.76 per cent in August.

Advancing lactation with these Jersey cows was seen to result in the lowest average tests during the second month after calving, with a gradual increase up to 5.55 per cent in the twelfth month. These average fat tests are shown in table 1. The actual seasonal range in fat percentages was 0.704 per cent (from 5.464 to 4.760), in contrast with 0.961 per cent (from 5.555 to 4.594) with advancing lactation, as depicted in figure 1. This suggests that under the relatively uniform climatic conditions of the Florida peninsula, the physiological factors exert a greater influence upon fat per-

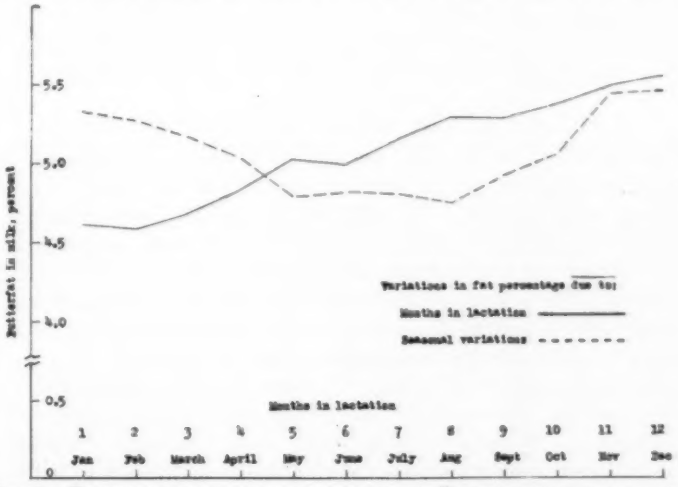



FIG. 1. THE AVERAGE BUTTERFAT PERCENTAGE IN JERSEY MILK, FROM A STUDY OF 293 RECORDS, INCREASES GRADUALLY FROM THE SECOND MONTH TO THE CLOSE OF LACTATION. IN PENINSULAR FLORIDA, THE SEASONAL TREND IN BUTTERFAT PERCENTAGE VARIES INVERSELY WITH THE TEMPERATURE, BEING HIGH IN WINTER AND LOW IN SUMMER.

centage in the milk of Jersey cows than do the environmental (seasonal) factors.

### STATISTICAL SIGNIFICANCE

Fisher's "Z" test (6) as modified by Snedecor (15), was used to test for significant contributions to variance in butterfat percentages in Jersey milk by season of the year and advancing lactation. The ratio of the between-month-after-calving mean square to the remainder mean square is 25.76 : 1. The ratio of the between-calendar-months mean square to the remainder mean square is 15.24 : 1. These two "F" values are much greater than the "F" values for the point of one per cent probability, as given in Snedecor's tables of "F" values. Both are highly significant. The ranges in fat percentage between months after calving are seen to be more highly significant, and of greater magnitude, than those for different calendar months. These relationships are shown in table 2.

TABLE 2

*Contribution of month-after-calving and of calendar-month to total variance in percentages of butterfat in the milk of Jersey cows*

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARES | "F" VALUE |
|---|---|---|---|---|
| Total | 144 − 1 = 143 | 30.832800 | | |
| Between months after calving | 12 − 1 = 11 | 15.272347 | 1.38839518 | 25.762 |
| Between calendar months | 12 − 1 = 11 | 9.039401 | .82176373 | 15.248 |
| Remainder | 11 × 11 = 121 | 6.521052 | .05389299 | |

### TEMPERATURE AFFECTS FAT PERCENTAGE

Meteorological data from the observatory of the United States Weather Bureau were assembled for the same period of years over which the butterfat records were obtained. The mean maximum and mean minimum temperatures were averaged, as shown in table 3, and used in analysing the relative influence of temperature upon butterfat percentage in the milk of Jersey cows. The method of analysing the butterfat percentages, as previously described, permitted the data used here to be practically independent of modifying influences due to advancing stage of lactation.

Between the range of 57 and 81° F., average mean temperatures for the different calendar months, there was a spread of approximately 0.70 per cent butterfat in the milk of these Jersey cows. Ezekial's formulae 8, 9 and 10 (5) were applied to these data, on the assumption that within this range of temperatures, the variation in fat percentage was a straight line function. For each increase of 10° F. between 57° and 81°, a decrease of 0.31 per cent fat was found. The results of this analysis are shown in figure 2, which is based upon data given in table 3.

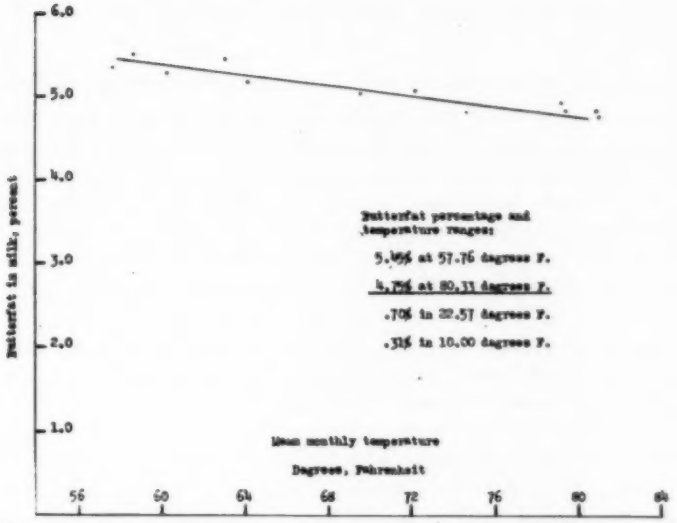



FIG. 2. ASSUMING THAT THE CHANGE OF BUTTERFAT PERCENTAGE WITH TEMPERATURE IS A STRAIGHT LINE FUNCTION WITHIN CERTAIN LIMITS, IT WAS CALCULATED FROM JERSEY BUTTERFAT TESTS ACCUMULATED OVER 16 YEARS, THAT A DECREASE OF 0.031 PER CENT FAT OCCURRED PER DEGREE INCREASE IN TEMPERATURE BETWEEN 57 AND 81°.

It may be mentioned in this connection that Hays (7) suggested from a study of fewer records of animals under more closely observed temperatures, but with the influence of advancing stage of lactation only partially

TABLE 3

*The relationship between mean temperatures, average rainfall and monthly butterfat percentages in Jersey milk at Gainesville, Florida, 1917 to 1932*

| MONTH | MEAN TEMPERATURE | | | AVERAGE RAINFALL INCHES | AVERAGE BUTTERFAT PERCENTAGE |
|---|---|---|---|---|---|
| | Maximum degrees | Minimum degrees | Average degrees | | |
| January | 68.7 | 46.5 | 57.6 | 3.15 | 5.33 |
| February | 71.7 | 48.7 | 60.2 | 2.83 | 5.27 |
| March | 75.4 | 52.8 | 64.1 | 3.62 | 5.17 |
| April | 81.4 | 57.4 | 69.4 | 3.23 | 5.03 |
| May | 86.5 | 62.5 | 74.5 | 3.46 | 4.80 |
| June | 89.7 | 68.8 | 79.25 | 6.96 | 4.83 |
| July | 90.6 | 70.8 | 80.7 | 9.07 | 4.81 |
| August | 90.7 | 70.8 | 80.75 | 6.26 | 4.76 |
| September | 89.0 | 69.1 | 79.05 | 5.36 | 4.92 |
| October | 82.9 | 61.2 | 72.05 | 2.56 | 5.06 |
| November | 74.6 | 51.4 | 63.0 | 1.94 | 5.44 |
| December | 69.5 | 47.6 | 58.55 | 2.59 | 5.49 |

eliminated, that a curvilinear function may appear at some point between 70 and 90° F.

### SEASON AND STAGE OF LACTATION, AS RELATED TO BREED AND LOCALITY

A search of the literature yielded six sets of butterfat tests from the Missouri (2, 14), Iowa (2) and Tennessee (18) stations and Sweden (11) in such form that the same method of analysis could be applied for variations due to season and stage of lactation. These data were for the Ayrshire, Guernsey, Holstein and Jersey breeds and for one large group of mixed breeds. A comparison is shown in table 4, of the results obtained by applying this method of analysis of variance to the data from these sources.

It may be noted that, in general, the low average butterfat test occurred during the second month after calving, with Holsteins, Guernseys and Jerseys, and in the third month with Ayrshires.

The low butterfat tests in the northern hemisphere occurred during the summer—June, July, or August. High tests were during the winter months of November, December, or January. The general trends of butterfat tests through the seasons and with advancing lactation under the Florida environment, are shown in figure 1.

Thus the general trends in fat percentage in the milk of Jersey cows in Florida are similar to those noted with the other breeds in more northerly latitudes. The magnitude of the variations in other latitudes differed with the several breeds, being greater as a rule with the breeds having a typically higher percentage of butterfat in the milk. In each instance where the records of a single breed were involved the range in variation of fat percentage, due to advance in stage of lactation, was significantly greater than that due to season of the year, as seen in the corresponding "F" values in table 4. Stating these results in other words, environment exerted less influence upon the fat percentage in cow's milk than did the internal or physiological factors due to advance in stage of lactation.

The only exception to the above generalization, seen in the groups of data analysed in table 4, is that of 240 records representing mixed breeds at the Missouri and Iowa stations. It is believed that the few numbers representing certain breeds, and the uneven distribution of the breeds within each cell, were sufficient to render the relative influences inconclusive because of the unequal weighting of breeds. Even in this instance, the fat percentages tended to follow the general trends of variation according to season and stage of lactation, as noted in the separate breed groups.

### DISCUSSION OF RESULTS

The butterfat content of Jersey milk ranged, with advancing lactation, between 4.594 per cent in the second month after calving, and 5.555 per cent

TABLE 4

*Comparison of stage of lactation and of season of the year, as these influence butterfat percentage in the milk of dairy cows in several regions*

| SOURCE | BREED | NUMBER | AVERAGE BUTTERFAT PERCENTAGE IN MONTHS AFTER CALVING | | | | | | | | | | | | "F" VALUES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| Florida | Jersey | 293 | 4.61 | 4.59 | 4.68 | 4.83 | 5.02 | 4.99 | 5.15 | 5.29 | 5.29 | 5.38 | 5.50 | 5.56 | 25.76 |
| Missouri | Jersey | 299 | 4.83 | 4.93 | 5.13 | 5.19 | 5.30 | 5.45 | 5.45 | 5.52 | 5.61 | 5.62 | 5.75 | 5.70 | 25.95 |
| | Holstein | 95 | 3.24 | 2.93 | 3.00 | 3.06 | 3.01 | 3.08 | 3.11 | 3.21 | 3.19 | 3.27 | 3.33 | 3.44 | 7.73 |
| United States | Guernsey | 3,763 | 4.63 | 4.59 | 4.71 | 4.84 | 4.97 | 5.08 | 5.16 | 5.21 | 5.29 | 5.39 | 5.49 | 5.59 | 488.75 |
| Missouri and Iowa | Mixed | 240 | 4.29 | 4.28 | 4.34 | 4.35 | 4.45 | 4.51 | 4.60 | 4.62 | 4.73 | 4.77 | 4.83 | 4.83 | 7.94 |
| Sweden | Ayrshire | 891 | 3.79 | 3.57 | 3.50 | 3.54 | 3.64 | 3.68 | 3.78 | 3.90 | 3.96 | 4.13 | 4.20 | 4.17 | 29.51 |
| | | | AVERAGE BUTTERFAT PERCENTAGE IN CALENDAR MONTHS | | | | | | | | | | | | |
| | | | Jan. | Feb. | March | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | |
| Florida | Jersey | 293 | 5.33 | 5.27 | 5.17 | 5.03 | 4.80 | 4.83 | 4.81 | 4.76 | 4.92 | 5.06 | 5.44 | 5.49 | 15.25 |
| Missouri | Jersey | 299 | 5.67 | 5.50 | 5.38 | 5.31 | 5.21 | 5.17 | 5.03 | 5.03 | 5.37 | 5.55 | 5.58 | 5.67 | 15.34 |
| | Holstein | 95 | 3.19 | 3.26 | 3.26 | 3.20 | 3.13 | 3.00 | 3.03 | 3.05 | 3.05 | 3.12 | 3.30 | 3.25 | 3.70 |
| United States | Guernsey | 3,763 | 5.22 | 5.17 | 5.13 | 5.07 | 5.01 | 4.95 | 4.89 | 4.88 | 5.02 | 5.15 | 5.22 | 5.24 | 70.48 |
| Missouri and Iowa | Mixed | 240 | 4.76 | 4.68 | 4.66 | 4.58 | 4.43 | 4.31 | 4.24 | 4.23 | 4.34 | 4.62 | 4.84 | 4.90 | 9.96 |
| Sweden | Ayrshire | 891 | 3.86 | 3.83 | 3.87 | 3.73 | 3.71 | 3.59 | 3.62 | 3.81 | 3.85 | 4.00 | 4.05 | 3.93 | 9.01 |

in the twelfth month after calving. The range for seasonal influence was between 4.760 per cent in August, and 5.464 per cent in December. By statistical analyses, these influences were measured separately in another way, by which the "F" values for variance were found to be 25.76 for between-months-after-calving, in contrast to 15.24 for between-calendar-months. ("Variance" is a term used by Fisher to denote the square of the standard deviation.) The arithmetical and analytical measures both indicate that the magnitude of physiological or internal influences affecting fat percentage in Jersey milk are appreciably greater than those exerted by the average external influences of environment existing in Florida. This observation is in accord with our analyses of the data obtained from separate breeds studied in more northerly latitudes.

## SUMMARY AND CONCLUSIONS

The seasonal trend in fat percentage in the milk of Jersey cows in the more uniform environment of Florida was found to vary almost inversely with temperature. The lowest average butterfat tests occurred as a rule in August and the highest in December.

The average test of Jersey milk in the first month after calving was 4.605 per cent, dropped to 4.594 in the second month, and then increased steadily to the twelfth month.

Advancing lactation exerted a greater influence than did season of the year upon the percentage of fat in the milk of Jersey cows.

An average increase of 10° F. between monthly mean temperatures of 57° and 81° resulted in an average decrease of 0.31 per cent butterfat in Jersey milk, in a study of records obtained over a 16-year period.

## ACKNOWLEDGEMENTS

Acknowledgements are made to Professor John M. Scott, formerly Animal Industrialist and Vice-Director of the Florida Agricultural Experiment Station, under whose supervision the records prior to May, 1928, were accumulated. Professor George W. Snedecor of Iowa State College, and Fred H. Hull and Bradford Knapp, Jr., of the Florida station, advised concerning the application of statistical methods. Completeness and accuracy of the records are credited to many persons employed in the care and management of the station dairy herd over this period of years.

## REFERENCES

(1) Baker, A. G., and H. T. Cranfield. Variations in composition of milk in certain midland districts of England during the years 1923–1931. The Jour. of Dairy Res. 4: 246–254. 1933.

(2) Eckles, C. H. Jahreszeitliche Schwankungen des prozentischen Fettgethaltes in Kühmilch. Milchw. Zentbl., Heft 11: 488–502. 1909.

(3) Eckles, C. H. Dairy cattle and milk production. Revised edition. 1923. The Macmillan Company, New York. Pages 406, 413.

(4) Eckles, C. H., and R. H. Shaw. The influence of the stage of lactation on the composition and properties of milk. U. S. Dept. Agr. Bur. An. Indus. Bul. 155: 1–88. 1913.

(5) Ezekial, M. Methods of correlation analysis. John Wiley & Sons, New York. 1930. Pages 51–64.

(6) Fisher, R. A. Statistical methods for research workers. Fourth revised edition. Oliver & Boyd, Edinburgh. 1933.

(7) Hays, W. P. The effect of environmental temperature on the percentage of fat in cow's milk. Journal of Dairy Science 9: 219–235. 1926.

(8) Headley, F. B. Effect of season on fat test and milk production of dairy cows. Nev. Agr. Exp. Sta. Bul. 131: 5–11. 1933.

(9) Hills, J. L. Variations in Milk. Vt. Agr. Exp. Sta. 9th Ann. Rpt.: 158–186. 1896.

(10) Hittcher, K. Untersuchung der Milch von sechzen Kühen. Landw. Jahrb. 23: 873–967. 1894.

(11) Hogstrom, K. A. Komjolkens fetthalt, dess normala växlinger och ärtlighet. (Swedish) Kungl. Landtbruks-Akad. Handl. och Tids.: 137–176. 1906.

(12) Linfield, F. B. Experiments with dairy cows. Utah Agr. Exp. Sta. Bul. 68: 167–237. 1900.

(13) Ragsdale, A. C., and S. Brody. The effect of temperature on the percentage of fat in milk. A first report. Journal of Dairy Science 5: 212–215. 1922.

(14) Ragsdale, A. C., and C. W. Turner. Seasonal variations in percentage of fat in cow's milk. Journal of Dairy Science 5: 544–554. 1922.

(15) Snedecor, G. W. Calculation and interpretation of analysis of variance and covariance. Collegiate Press Inc., Ames, Iowa. Pages 1–96. 1934.

(16) Spier, J. Milk records. Second season—giving the yield of milk and butterfat of 389 cows. Trans. Highland Agr'l. Soc. Scot. 5th series, 17: 182–212. 1905.

(17) Weaver, E., and C. A. Mathews. The influence of temperature and certain other factors upon the percentage of fat in milk. Iowa Agr. Exp. Sta. Res. Bul. 107: 158–180. 1928.

(18) Wylie, C. E. The effect of season on the milk and fat production of Jersey cows. Journal of Dairy Science 8: 127–131. 1925.

# BEHAVIOR OF CASEINATE SOLS IN A STUDY OF A HYSTERESIS-LIKE PHENOMENON IN THE RENNET COAGULATION OF HEATED MILK*

MILTON E. POWELL AND L. S. PALMER

*Division of Agricultural Biochemistry, University of Minnesota, St. Paul*

It is commonly known that heated milk does not react normally with rennet. That heat treatment is not always detrimental was first observed by Stassano and Talarico (1), who noticed that milk heated to 65° C. coagulates more rapidly than raw milk. Similar observations were made by Rupp (2), and Mattick and Hallett (3), the latter investigators also observing an apparent hysteresis-like phenomenon.[1] They found that when milk is heated for 30 minutes at 105° F. to 141° F. and rapidly cooled to 84° F. the first effect is increased coagulability, but the milk gradually loses coagulability as the interval between heating and addition of rennet lengthens. When the milk is heated above 145° F. the loss of coagulability is immediate and continues to increase as rennet addition is delayed, reaching a maximum after 5 hours. Moir (4) made a similar observation with flash pasteurized milk.

As Hammarsten (5) early demonstrated that only the calcium caseinate and calcium phosphate systems in milk are involved in the clotting by rennet, it appeared feasible to employ these two components as an artificial "milk" in the study of the hyteresis-like phenomenon of heated milks. It was hoped that some evidence could be secured as to the analogous fraction in natural milk responsible for the different behavior of heated milks toward rennet.

## COAGULABILITY OF HEAT-TREATED CALCIUM CASEINATE-COLLOIDAL CALCIUM PHOSPHATE "MILKS"

All of the "milks" were prepared to contain 2.5 per cent casein in order to approximate the quantity of casein in natural milk. The casein was prepared by the method of Van Slyke and Baker (6) as refined by Van Slyke (7). The concentration of calcium associated with the casein can be varied through a wide range as Porcher (8) has shown. By assuming that casein is combined with 1.15 per cent Ca at the pH of milk [Palmer and Richardson (9)] and that casein contains 0.85 per cent P [Hammarsten

Received for publication March 4, 1935.

* Condensed from the thesis of Milton E. Powell submitted in partial fulfillment of the requirements for the degree of Ph.D., University of Minnesota. All pertinent data are available in the Division of Agricultural Biochemistry, University of Minnesota. Paper No. 1338, Journal Series, Minnesota Agricultural Experiment Station.

[1] Hysteresis is a term applied to colloidal systems that react according to the past treatment which they have received.

(10), Lehmann (11), Van Slyke (12)] the data on Van Slyke and Bosworth (13) may be recalculated to show that on the average 30 per cent of the total P and 43 per cent of the total Ca in natural milk occurs as colloidal mineral compounds. However, in the preparation of the artificial "milks" only about 77 per cent of these quantities were used, otherwise the system would coagulate spontaneously when warmed to 40° C.

The calcium caseinate-colloidal calcium phosphate complexes were prepared by triturating the casein in a small quantity of clear limewater; subsequent small additions of limewater were made until a thin paste was formed, whereupon a sufficient amount was then added to prepare an alkaline caseinate of pH about 10.5. After allowing this solution to stand for about an hour to insure complete peptization of the casein the pH was reduced to that of natural milk by the slow addition of 0.6 per cent $H_3PO_4$ solution from a capillary-tipped pipette extending beneath the surface while the liquid was rapidly agitated by a motor driven stirrer. After dilution to the required volume with distilled water the "milk was aged for 14 hours at 5° C. The following data illustrate a typical "milk": 25 gm. casein, 860 cc. clear limewater, 85 cc. $H_3PO_4$ solution, 65 cc. $H_2O$; pH 6.65. Eight different lots of "milk" were prepared by this method.

Later another method of preparation was adopted in which the pH did not exceed the limits of normal milk at any time. These systems produced coagulation curves similar to those prepared as above. A 5 per cent calcium caseinate sol was first prepared by triturating the casein in the usual way and adding sufficient limewater to produce a pH comparable to natural milk. After diluting the sol to about a 5 per cent casein content it was aged 14 hours at 5° C. Next a predetermined amount of limewater and 0.6 per cent $H_3PO_4$ solution were slowly added simultaneously to the mechanically stirred caseinate sol from capillary-tipped pipettes. The acid solution was first diluted with sufficient distilled water to increase the complete complex to the desired volume. An example is 25 gm. casein, 345 cc. clear limewater, 150 cc. water; pH 6.72. To this aged sol were then added as described 300 cc. clear limewater, 65 cc. of 0.6 per cent $H_3PO_4$ solution diluted with 140 cc. distilled water; pH 6.70. The "milk" was aged 14 hours at 5° C. before using.

Since only heat-treated portions not coagulated by the heat treatment itself were desired a sample of each "milk" was heated at 85° C. or higher for 10 minutes, cooled and observed in an ultramicroscope. Any clustering observed in the collodial suspension could usually be prevented by a short dialysis of the unheated "milk." The heating of the artificial "milks" was carried out as follows: Two portions of each "milk" were placed in loosely stoppered flasks; one flask was rapidly heated in a water bath to 60° C., the other to 85° C. and maintained at the respective temperatures for 30 minutes. Both portions were then promptly cooled to 35° C. and

samples removed for immediate coagulation; the remainder was cooled to approximately 20° C. Samples of the latter were tested for rennet coagulability at 40, 70 and 130 minute intervals after the end of the heating period. All coagulation tests were made at 40° C.

As a result of the heat-treatment at 60° C. and 85° C. the pH of the "milks" decreased slightly, the greatest lowering always occurring in the

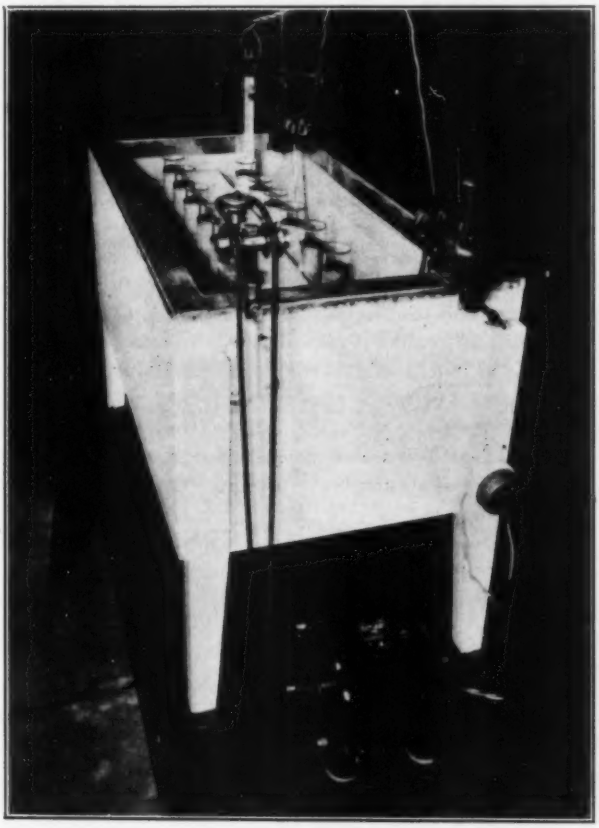


FIG. 1. Electrically heated and controlled water bath in which all samples were coagulated.

portion heated at 85° C. However, in no case was the decrease greater than pH 0.05 and usually only pH 0.03 as the typical example in Table 1, Exp. A indicates. In occasional experiments pH measurements were made on the control and the heated portions at the beginning and again at the completion of the experiment. Data are given on this point in the following

typical example, the first values given being the initial pH and the second the pH two and one-half hours later, at the completion of the experiment: unheated calcium caseinate-phosphate "milk" pH 6.70 and 6.70; after heating to 60° C. pH 6.66 and 6.662; after heating to 85° C. pH 6.65 and 6.65.

Fig. 1 shows the electrically heated water bath in which all samples were coagulated. In this figure are visible the thermo-regulator, heating element, stirrer and the series of copper tubes containing the 100 cc. glass coagulation tubes. The latter are fitted with glass-tipped rubber tubing that will extend through an opening in the bottom of the bath; also a rubber stopper that will close the opening in the bottom of the bath thus permitting a water contact between the glass and copper tubes. Each tube is closed with a screw clamp close to the bottom of the bath.

Fifty cc. samples of "milk" were pipetted into the coagulation tubes 5 minutes before the rennet was added; a predetermined amount of $CaCl_2$ solution was put in next and the mixture stirred occasionally. As soon as the "milk" had reached the temperature of the bath rennet was added, a stop-watch started and the mixture stirred for 30 seconds. The quantities of $CaCl_2$ solution and rennet[2] were adjusted to cause coagulation of the unheated controls in 3 to 5 minutes; these same quantities were added to the heated portions. The screw clamp was opened slightly at the end of 3 minutes and a fine stream of "milk" allowed to run into a glass beaker. As soon as flakes of curd were visible on the side of the beaker the watch was stopped and the coagulation time recorded. All experiments were carried out in duplicate.

With some of the unheated "milks" the concentrations of $CaCl_2$ solution and rennet employed produced merely a coagulation within the desired time whereas with others the "milk" clotted although no attempt was made to insure clots. The diluted rennet solution was held in very cold water so as to reduce inactivation to a minimum. A typical coagulation curve of a heat-treated calcium caseinate-phosphate complex is shown in Fig. 2A. The curve indicates that this system exhibits a hysteresis-like phenomenon when heated to 60° C. and 85° C. The pH of the original "milk" was 6.82; after heating to 60° C. pH 6.80 and after heating to 85° C. pH 6.785.

*Effect of Heat-treatment on Casein.*—According to Marui (14), the effects of heating caseinate solutions are not deep-seated, for he prepared an artificial "milk" which coagulated normally with rennet by using casein isolated from a caseinate solution which had been boiled. We performed a similar experiment. A calcium caseinate-phosphate "milk" was first prepared using 100 gm. casein, 3600 cc. clear limewater, 300 cc. 0.6 per cent. $H_3PO_4$ solution; final volume 4000 cc., pH 6.61. This "milk" required

[2] Hansen's Liquid Cheese Rennet was used as a stock solution.

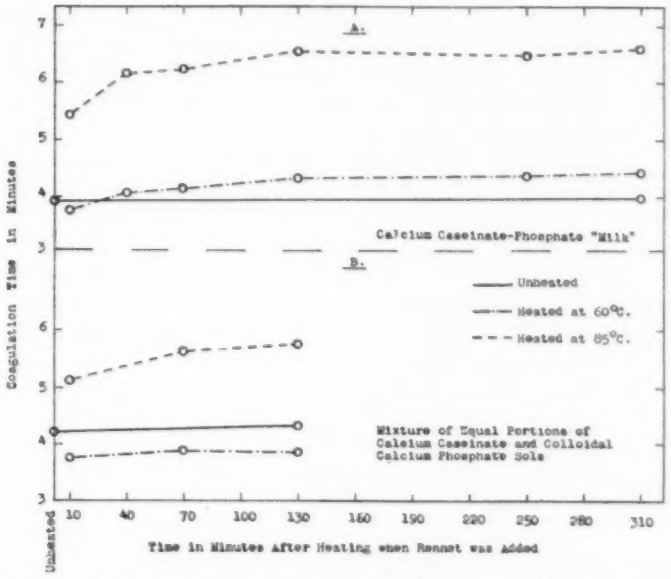

A.
Calcium Caseinate-Phosphate "Milk"
B.
Unheated
Heated at 60°C.
Heated at 85°C.
Mixture of Equal Portions of Calcium Caseinate and Colloidal Calcium Phosphate Sols
Coagulation Time in Minutes
Unheated
10 40 70 100 130 160 190 220 250 280 310
Time in Minutes After Heating when Rennet was Added

FIG. 2. A. Typical coagulation curves of a calcium caseinate-colloidal calcium phosphate "milk" coagulated at intervals after heating at 60° C. and 85° C. for 30 min. B. Typical coagulation curves of mixture of calcium caseinate and colloidal calcium phosphate sols coagulated at intervals after heating at 60° C. and 85° C. for 30 min.

dialysis before it would withstand boiling without showing clustering in the ultramicroscope; the pH after dialysis was 6.80. The product was next held in a boiling water bath for 30 minutes, cooled and aged 14 hours at 5° C. The casein was then isolated by the method of Van Slyke and Baker (6) and Van Slyke (7). Using this casein a new calcium caseinate-phosphate "milk" was prepared with 26.5 gm. casein, 950 cc. limewater and 76 cc. 0.6 per cent $H_3PO_4$ solution; final volume 1060 cc., pH 6.72. A boiled sample was free from ultramicroscopic clustered particles. The coagulation times of the control and portions heated to 60° C. and 85° C. are given in Table 1, Exp. A, and show the same general trend as the "milks" shown in Fig. 2.

### COAGULABILITY OF MIXTURES OF CALCIUM CASEINATE AND COLLOIDAL CALCIUM PHOSPHATE SOLS

A representative calcium caseinate sol was prepared by triturating 25 gm. casein in 422 cc. clear limewater. A dense milky sol resulted which was diluted to 500 cc. with distilled water; final pH 6.75. A colloidal calcium phosphate sol was prepared by dissolving 5 gm. of high grade gelatin in

TABLE 1

*Effects of heating various artificial "milks" on their coagulation with rennet*

| EXP. NO. | TYPE OF SYSTEM | FRACTION HEATED | HEATING TEMP. | pH OF HEATED FRACTION | COAGULATION TIME AT VARIOUS PERIODS AFTER HEATING | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0 | 10[1] | 40 | 70 | 130 | 190 |
| | | | °C. | | min. | min. | min. | min. | min. | min. |
| A. | Calcium Caseinate-Phosphate | Entire "Milk" | Unheated | 6.72 | 3:20<br>3:23 | | | | 3:19 | |
| | | | 60 | 6.70 | | 3:14<br>3:15 | 3:20<br>3:28 | 3:35<br>3:38 | 3:41<br>3:44 | |
| | | | 85 | 6.69 | | 5:00<br>5:05 | 5:34<br>5:35 | 5:50<br>5:51 | 6:20<br>6:23 | |
| B. | Calcium Caseinate | Calcium Caseinate | Unheated | 6.72 | 4:00<br>4:01 | | | | 3:54<br>3:58 | |
| | Calcium Phosphate Mixture | | 60 | 6.71 | | 4:06<br>4:00 | | 3:58<br>3:55 | 3:50<br>3:47 | |
| | | | 85 | 6.70 | | 4:03<br>4:06 | | 3:57<br>4:00 | 3:53<br>3:55 | |
| C. | Calcium Caseinate | Calcium Phosphate | Unheated | 6.60<br>(6.69)[2] | 4:49<br>4:50 | | | | | 4:55 |
| | Calcium Phosphate Mixture | | 60 | 5.94<br>(6.59)[2] | | 5:00<br>5:00 | | | 4:56<br>5:02 | 5:00<br>5:04 |
| | | | 85 | 5.71<br>(6.55)[2] | | 4:29<br>4:24 | | | 4:26<br>4:22 | 4:23<br>4:22 |
| D. | Calcium Caseinate | Entire Sol | Unheated | 6.70 | 4:02<br>4:05 | | | | 4:04<br>4:06 | |
| | | | 60 | 6.70 | | 4:05<br>4:07 | 4:05<br>4:05 | 4:10<br>4:06 | 4:12<br>4:07 | |
| | | | 85 | 6.70 | | 4:15<br>4:15 | 4:18<br>4:15 | 4:15<br>4:19 | 4:17<br>4:18 | |
| E. | Calcium Caseinate-Arsenate | Entire "Milk" | Unheated | 6.75 | 4:20<br>4:21 | | | | 4:22<br>4:21 | |
| | | | 60 | 6.72 | | 4:02<br>3:59 | 4:11<br>4:12 | 4:14<br>4:15 | 4:10<br>4:10 | |
| | | | 85 | 6.73 | | 4:00<br>3:55 | 3:49<br>3:50 | 3:45<br>3:46 | 3:45<br>3:44 | |
| F. | Calcium Caseinate-Oxalate | Entire "Milk" | Unheated | 6.84 | 3:45<br>3:45 | | | | 3:45<br>3:45 | |
| | | | 60 | 6.86 | | 3:45<br>3:45 | 3:45<br>3:45 | 3:45<br>3:45 | 3:45<br>3:45 | |
| | | | 85 | 6.86 | | 3:45<br>3:45 | 3:45<br>3:45 | 3:45<br>3:45 | 3:45<br>3:45 | |

[1] Approximately 10 minutes were required to cool the heated "milks" and adjust the first samples to the temperature of the water bath.

[2] pH of a mixture containing equal portions of unheated caseinate and heated phosphate sols.

430 cc. clear limewater, cooling the solution under tap water and partially neutralizing 56.5 cc. 0.6 per cent $H_3PO_4$ solution with some of the gelatin-limewater solution until a slight precipitate began to form. This limewater-acid mixture was slowly added to the remainder of the mechanically stirred gelatin-limewater solution from a capillary-tipped pipette held beneath the surface. A stable colloidal suspension resulted which was diluted to 500 cc. with distilled water; pH 6.60.

*Heat-treating the Mixture.*—A mixture of equal portions of the calcium caseinate and calcium phosphate sols appeared like a calcium caseinate-calcium phosphate complex; the pH was 6.71. The coagulation curves of this mixture before and after heating at 60° C. and 85° C. are shown in Fig 2B and are similar to those of the complex. The pH of the portion after heating to 60° C. was 6.68; after heating to 85° C. pH 6.658.

*Heat-treating the Calcium Caseinate Fraction.*—In order to determine whether the hysteresis effects of heating involve the protein fraction or the inorganic phosphates or whether the presence of both is required a series of calcium caseinate sols were prepared as previously described and also a parallel series of colloidal calcium phosphate sols. The phosphate sols were made by adding calcium sucrate and $H_3PO_4$ solutions simultaneously to a dilute gelatin solution. A typical sol consisted of 420 cc. 1.2 per cent gelatin solution, 10 cc. of a calcium sucrate solution containing 8.0 per cent $Ca(OH)_2$ and 70 cc. 0.6 per cent $H_3PO_4$ solution. Both the sucrate and acid solutions were diluted to 150 cc. with some of the gelatin solution and were then slowly added to the remainder of the mechanically stirred gelatin from capillary-tipped pipettes held beneath the surface of the liquid. The stable translucent sol had a pH 6.72.

In order to coagulate 50 cc. of a mixture of equal portions of caseinate and phosphate sols 1.0 cc. of 0.15N $CaCl_2$ solution was required in addition to the rennet. In one series of experiments one-half of the necessary $CaCl_2$ was added to the calcium caseinate sol before heating, but in the other series the $CaCl_2$ was added to the mixed sols immediately before the coagulation test. Table 1, Exp. B shows the effects on coagulability with rennet when the calcium caseinate fraction was heated to 60° C. and 85° C. for 30 minutes before cooling and adding the colloidal calcium phosphate sol. The results show no significant effect on the coagulation time of the mixed sols.

*Heat-treating the Calcium Phosphate Fraction.*—Calcium caseinate and colloidal calcium phosphate sols were prepared in a manner similar to those just described. In this study the phosphate sols were previously heated and cooled before mixing with the calcium caseinate fraction for coagulation. Nine separate experiments were conducted in which four different lots of casein and calcium sucrate solutions were used. A typical experiment follows: A 5 per cent calcium caseinate sol made with 30 gm. casein and 415 cc. clear limewater had a pH of 6.80 after diluting to 600 cc. The phosphate sol was made with 485 cc. 1.24 per cent gelatin, 19.75 cc. calcium sucrate containing 5.3 per cent $Ca(OH)_2$ and 94 cc. 0.6 per cent $H_3PO_4$ solutions. Both the acid and base solutions were diluted to 150 cc. with some of the gelatin solution and then were added simultaneously to the remainder of the gelatin; pH 6.60.

All cases in which the colloidal calcium phosphate sols were heated a peptization[3] of colloidal material resulted, being especially marked at 85° C. The hydrogen-ion concentration was always increased by heat-treating these sols, the extent of the increase being directly proportional to the temperature. For example, in the sol just described after heating at 40° C. the pH was 6.37; at 60° C., 5.94 and at 85° C. 5.71. Similar but much less extensive effects were obtained after mixing the raw caseinate sol and the heated phosphate sols as shown in Table 1, Exp. C, (pH data in parentheses). This experiment shows the effects on coagulation when portions of the phosphate sol were heated at 60° C. and 85° C. for 30 minutes before cooling and adding the caseinate for rennet coagulation. The hysteresis-like effects of delayed rennet addition are absent. Although this experiment is representative of most of those performed, in two trials in which different lots of casein and calcium sucrate were used to prepare the respective sols a decreased coagulability was observed which was so marked that it was apparent even when the phosphate fraction was heated at 40° C. and no coagulation curves were obtainable. The explanation has not yet been found for these two results.

### COAGULABILITY OF HEAT-TREATED CALCIUM CASEINATE SOLS

Several investigators, including one of us, (15) have postulated that the effects of heating milk on its coagulation with rennet are to be explained on a basis of a disturbance in the colloidal calcium caseinate system. In order to determine whether artificial systems of calcium caseinate would exhibit the hysteresis-like behavior portions of several calcium caseinate sols were heated to 60° C. and 85° C. for 30 minutes, cooled and coagulated at 40° C. In some sols a portion of the $CaCl_2$ required for coagulation was added before heating while in others it was added afterwards; this modification did not affect the coagulation times.

The caseinate systems produced a peculiar type of clot with rennet, the curd having a gummy or rubbery consistency which synerized rapidly. Also, considerably greater concentrations of $CaCl_2$ were necessary than for the caseinate-phosphate complexes. One sol clotted with one cc. 0.35N $CaCl_2$ solution but others required one cc. 0.48N $CaCl_2$. Table 1, Exp. D shows the typical behavior of the heated sols of calcium caseinate. Previous heating of these sols was practically without effect on their coagulability, there being no evidence of the hysteresis shown by the caseinate-phosphate complexes.

[3] Although the decreased colloidality was found to be closely associated with the increase in acidity, the previous colloidality could not be restored by addition of alkali to the previous pH. Example: Original pH of phosphate 6.81; after heating 85° C. 5.78; after addition of alkali 6.76. The presence of soluble electrolytes was found to be without influence inasmuch as dialysis failed to prevent both the decrease in colloidality and the drop in pH on heating.

### COAGULABILITY OF CALCIUM CASEINATE COMPLEXES MADE WITH ARSENIC AND OXALIC ACIDS

Although Hammarsten (16) first stated that other acids should replace phosphoric in the preparation of artificial "milks" this hypothesis was developed by Porcher (8) and Marui (14). The former postulated that arsenic acid could be used on account of its periodic relationship to phosphoric. Accordingly, a few calcium caseinate-calcium arsenate "milks" were prepared and studies made of the coagulation of heated portions. These "milks" were prepared in general by triturating casein in limewater to pH about 10.50 and increasing the hydrogen-ion concentration by the slow addition of M/10 $H_3AsO_4$ solution. A representative "milk" contained 30 gm. casein, 1020 cc. clear limewater and 110 cc. M/10 $H_3AsO_4$ solution; after dilution to 1200 cc. the final pH was 6.68. A preliminary dialysis was necessary to prevent coagulation at 85° C.; the pH after dialysis had increased to 6.75. Although the "milk" was now stable at 85° C. it coagulated in boiling water.

These "milks" had the same appearance as the caseinate-phosphate complexes but required more $CaCl_2$ solution as 50 cc. of "milk" required one cc. N/2 $CaCl_2$ solution for rennet coagulation at 40° C., thus resembling the calcium caseinate sols. Portions of the "milk" were heated to 60° C. and 85° C. for 30 minutes, cooled to 20° C. and the $CaCl_2$ solution added. Table 1, Exp. E shows the coagulation data of the above typical arsenate "milk"; no significant hysteresis was observed on holding subsequent to the heat treatments.

Two unsuccessful attempts were made to prepare a colloidal calcium arsenate sol in gelatin by using calcium sucrate and M/10 $H_3AsO_4$ solution. A heavy precipitate is first formed but as the addition of acid increases and the pH of the mixture approaches that of natural milk the precipitate disappears and at the pH 6.67 the solutions are nearly transparent. Evidently a colloidal calcium arsenate does not exist at the pH of natural milk. This undoubtedly explains why the $CaCl_2$ requirements of the calcium caseinate-arsenate "milks" were similar to those of the pure calcium caseinate sols.

Marui found that when sufficient $CaCl_2$ was added to a solution of casein in sodium acetate-oxalate it became milky and would coagulate with rennet. We extended this observation to determine whether or not a complex of calcium caseinate and collodial calcium oxalate would exhibit a hysteresis when heated and subsequently coagulated with rennet at 40° C. These "milks" were prepared in the general manner already described. A typical example is as follows: 30 gm. casein, 1020 cc. limewater and 95 cc. M/10 oxalic acid slowly added with the aid of mechanical stirring; pH after dilution to 1200 cc. was 6.84.

These systems, like the arsenate "milks" and the caseinate sols, required more $CaCl_2$ than the caseinate-phosphate complexes; in all cases it was

added after heating. In order to coagulate the above "milk" in the desired time one cc. 0.35N $CaCl_2$ solution was required for each 50 cc. Table 1, Exp. F shows the coagulation times of the calcium caseinate-collodial calcium oxalate "milk" described above after heating to 60° C. and 85° C. for 30 minutes. No hysteresis is apparent, the heating being without effect on the rate of coagulation.

### CATAPHORESIS OF HEATED SOLS OF CALCIUM CASEINATE AND CALCIUM CASEINATE-CALCIUM PHOSPHATE "MILK" [4]

Richardson and Palmer (17) have shown that slightly acid calcium caseinate sols exhibit an increased rate of migration in an electric field after heating in boiling water. It was hoped that the hysteresis-like phenomenon might also be followed by cataphoretic measurements and preliminary observations were made by the Northrup-Kunitz method of electrophoresis. The details of this method are discussed by Moyer (18) and will not be included here. With this method the individual particles are observed in a dark field and the average of 10 observations at both "stationary levels" are used for the calculation of the velocity of migration. The velocities are expressed in μ/sec./volt/cm.

The great dilution of the sol necessary for observation fosters a peptization of the particles, especially if a sodium buffer is used. Therefore, a calcium buffer was prepared by adding clear limewater to dilute $H_3PO_4$ solution until a stable pH of 6.67 was reached. The clear liquid was carefully siphoned off and supercentrifuged at 40,000 R.P.M. Only a very few particles were visible in the ultramicroscope.

A 5 per cent calcium caseinate sol was prepared by triturating 2.5 gm. casein in 45 cc. limewater and diluting to 50 cc. A calcium caseinate-phosphate complex was prepared with 2.5 gm. casein, 93 cc. limewater and 9 cc. 0.6 per cent $H_3PO_4$ solution; the resulting pH was 6.61. Both sols were aged 14 hours at 5° C. and then lightly centrifuged before using. The calcium caseinate sol was heated to 85° C. for 30 minutes while portions of the caseinate-phosphate complex were heated to 60° C. and 85° C. for 30 minutes and then cooled immediately to 20° C. The velocity of the particles was measured at 10, 40, and 70 minutes after heating. Table 2 shows that the effects of heating on the velocity of migration in an electric field are more pronounced on the calcium caseinate-phosphate complex than on the calcium caseinate sol.

### DISCUSSION

From the experimental findings it is apparent that those components in milk which are involved in the clotting phenomenon in the presence of

[4] Direction and assistance in this phase of the work was rendered by Dr. L. S. Moyer, National Research Council Fellow in the Division of Agricultural Biochemistry, University of Minnesota.

TABLE 2

*Effects of heating a calcium caseinate sol and a calcium caseinate-phosphate "milk" on their migration in an electric field*

| TYPE OF SYSTEM | HEATING TEMPERATURE | VELOCITY OF MIGRATION IN μ/SEC./VOLT/CM. AT VARIOUS PERIODS AFTER HEATING | | | |
|---|---|---|---|---|---|
| | | 0 | 10[1] | 40 | 70 min. |
| | °C. | | | | |
| Calcium Caseinate | Unheated | 1.42 | | | |
| | 85 | | 1.54 | 1.46 | |
| or | | | | | |
| Calcium Caseinate-Phosphate | Unheated | 1.36 | | | |
| | 60 | | 1.72 | 1.70 | 1.63 |
| | 85 | | 1.72 | 1.72 | 1.74 |

[1] Approximately 10 minutes were required to cool and prepare the first samples for observation.

rennet are likewise responsible for the hysteresis-like effect exhibited by heated milks when coagulated at definite time intervals after heating. Using coagulation as a criterion, since no special effort was made to secure clots, it was found that heated artificial systems of calcium caseinate-colloidal calcium phosphate show a progressive loss of coagulability with age.

It is the opinion of György (19), Kometiani (20) and Pyne (21) that the calcium caseinate and colloidal calcium phosphate systems in natural milk exist in a chemical union. If this opinion is correct a like association must exist to a certain extent in the artificial complex. Nevertheless, our results have shown that such a union is not essential either for coagulation by rennet or for the accompanying hysteresis, inasmuch as equal portions of the separate sols of calcium caseinate and colloidal calcium phosphate when mixed before heating produced a coagulation and hysteresis similar to that of the complex. The artificial systems even exhibited some evidence of the accelerating effect of low temperature heating on rennet coagulability which has been observed for natural milk.

The effects of heat upon the calcium caseinate fraction appear much less significant than previously suggested. Although ultramicroscopic observations revealed some slight physical change toward a coarser colloidal dispersion this effect was not registered by coagulability or hysteresis. Even boiling an artificial caseinate-phosphate "milk" failed to impare the coagulability of new "milks" prepared with casein isolated from the heated complex. Likewise when the colloidal calcium phosphate fraction was heated before mixing with the caseinate the effects of delayed addition of rennet were *nil* although minor differences in heating temperature were evident. The presence of both the caseinate and phosphate during the heating process is essential for the hysteresis-like phenomenon.

The decrease in pH of the heated phosphate sols is greatly masked by the presence of caseinate when the two sols are mixed in equal portions. This is true even to a greater degree when both fractions are present throughout the heat-treatment. Such extreme changes in hydrogen-ion concentration might mitigate other effects of heat such as a loss of much colloidal phosphate material. The peptization of colloidal calcium phosphate observed is contrary to the existing beliefs of the effects of heat on the calcium salts in natural milk.

It is not to be inferred that the mere presence of a second colloid is the criterion for the hysteresis behavior in the heated systems. We produced coagulation in a calcium caseinate-colloidal calcium oxalate "milk" as did Marui (14), but these complexes showed no hysteresis-like behavior after heating. It appears that colloidal calcium phosphates are essential for this phenomenon.

The preliminary cataphoretic studies of the heated calcium caseinate-phosphate complex and the heated caseinate sol indicate that the hysteresis phenomenon cannot be followed by changes in the velocity of migration of the colloidal particles. The effects observed seem to be somewhat contrary to those obtained by Richardson and Palmer (17) when using a modified Burton cataphoretic tube with raw and heated calcium caseinate sols. However, the experimental conditions of the present investigation are not strictly comparable to those of the former so that final judgement should be reserved. Nevertheless, it is indicated here that the effects of heat treatment on the cataphoretic velocity of rennet coagulable sols are not so closely related to their coagulability as the experiments of Richardson and Palmer suggested.

Our experiments cannot be interpreted as disproving the belief of Moir (4) regarding the rôle of a coagulation of lactalbumin in natural milk in connection with the phenomenon investigated. However, they suggest that Moir's findings are not of major importance in explaining it for natural milk.

## CONCLUSIONS

1. Heated calcium caesinate-colloidal calcium phosphate "milks" as well as mixtures of equal parts of the two separate sols exhibit a hysteresis-like behavior when coagulated with rennet at definite intervals after heating.

2. Heat-treatment of calcium caseinate sols is practically without effect on their rate of coagulation either alone or when mixed with colloidal calcium phosphate.

3. Heat-treatment of the colloidal calcium phosphate portion before adding the caseinate is practically without effect on the rate of coagulation of the mixture.

4. The hysteresis effects of heat on the complex result from the presence of both the caseinate and phosphate during heat-treatment.

5. A complex containing colloidal calcium oxalate exhibits no hysteresis as a result of heating.

6. A colloidal calcium arsenate does not exist at the pH of natural milk.

7. Cataphoretic studies do not appear applicable as a means of following the hysteresis; additional work should be done, however, before discarding it. The fact that heating caused a greater increase in velocity of migration of a caseinate-phosphate complex than a calcium caseinate sol shows that the effects of heat are greatest in the presence of the colloidal phosphates.

## REFERENCES

(1) STASSANO, H., AND TALARICO, J. De l'influence de la cuisson sur la caséification du lait par le lab-ferment. Compt. rend. soc. biol. **69**: 254. 1910.

(2) RUPP, P. Chemical changes produced in cows' milk by pasteurization. U. S. Dept. Agr. Bur. Animal Ind. Bull. 166. 1913.

(3) MATTICK, E. C. V., AND HALLETT, H. S. The effect of heat on milk. Jour. Agr. Sci. **19**: 452. 1929.

(4) MOIR, G. M. The effect of heat upon the rennin coagulation. I. Jour. Dairy Res. **2**: 68. 1930. II. Jour. Dairy Res. 3: 80. 1931.

(5) HAMMARSTEN, O. Ueber die Milchgerinnung und die dabei wirkenden fermente der Magenschleimhaut. Jahrsber. f. Tierchem. **2**: 118. 1874.
Ueber den chemischen Verlauf bei der Gerinnung des Caseins mit Lab. Jahrsber. f. Tierchem. **4**: 135. 1875.

(6) VAN SLYKE, L. L., AND BAKER, J. C. The preparation of pure casein. Jour. Biol. Chem. **35**: 127. 1918.

(7) VAN SLYKE, L. L. Chemistry of casein. Chem. Age **32**: 163. 1924.

(8) PORCHER, CH. Le lait au point de vue colloidal. Le Lait, Lyon. 1929.

(9) PALMER, L. S., AND RICHARDSON, G. A. The colloid chemistry of rennet coagulation. Third Coll. Symp. Mono. Chemical Catalog Co. New York. 1925.

(10) HAMMARSTEN, O. Zur Frage, ob das Casein ein einheitlicher Stoff sei. Zeit. Physiol. Chem. **7**: 227. 1883.

(11) LEHMANN, J. See HEMPEL, W. Die Milchuntersuchungen Professor Dr. Julius Lehmann's. Arch. f. d. ges. Physiol. **56**: 558. 1894.

(12) VAN SLYKE, L. L. The chemistry of casein. Proc. World's Dairy Cong. 1923. **2**: 1145. U. S. Dept. Agr. Govt. Print. Off. Washington, D. C.

(13) VAN SLYKE, L. L., AND BOSWORTH, A. W. Condition of casein and salts in milk. Jour. Biol. Chem. **20**: 135. 1915.

(14) MARUI, S. Untersuchung über das Labferment. Biochem. Zeit. **173**: 363–388. 1926.

(15) PALMER, L. S. The effect of heat on the calcium salts and rennet coagulability of cows' milk. Proc. Soc. Exp. Biol. and Med. **19**: 137. 1921.

(16) HAMMARSTEN, O. Zur Kenntniss des Caseins und der Wirkung des Labfermentes. Jahrsber. f. Tierchem. **7**: 158. 1878.

(17) RICHARDSON, G. A., AND PALMER, L. S. Rennin action in relation to electrokinetic phenomena. Jour. Phys. Chem. **33**: 557. 1929.

(18) MOYER, L. S. Species relationships in *Euphorbia* as shown by the electrophoresis of latex. Amer. Jour. Botany **21**: 293. 1934.

(19) GYÖRGY, P. Zur Verteilung des Calciums und des anorganischen Phosphors in der Milch. Biochem. Zeit. **142**: 1. 1923.

(20) KOMETIANI, P. A. Veränderung einiger Milchbestandteile durch Erhitzen. Milchw. Forsch. **12**: 433. 1931.

(21) PYNE, G. T. The determination of milk proteins by formaldehyde titration. Biochem. Jour. **26**: 1006. 1932.

The colloidal phosphate of milk. Biochem. Jour. **28**: 940. 1934.

## American Dairy Science Association Announcements

### COMMITTEES OF THE MANUFACTURING SECTION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION, 1935

*Committee on National Intercollegiate Dairy Products Judging Contest:*

H. W. Gregory (Purdue), Chairman
R. B. Stoltz (Ohio)
Wm. White (U. S. D. A.)
P. A. Downs (Nebraska)
R. W. Smith, Jr. (Vermont)

*Committee on Chemical Methods for the Analysis of Milk and Dairy Products:*

E. S. Guthrie (Cornell), Chairman
H. A. Ruehe (Illinois)
L. C. Thomsen (Wisconsin)
G. H. Wilster (Oregon)
W. H. Martin (Kansas)
W. D. Swope (Pennsylvania)
F. J. Doan (Pennsylvania)
B. L. Herrington (Cornell)

*Subcommittee on Milk and Cream:*

H. A. Ruehe (Illinois), Chairman
R. W. Bell (U. S. D. A.)
E. W. Bird (Iowa)

*Subcommittee on Butter:*

L. C. Thomsen (Wisconsin), Chairman
D. H. Nelson (California)
S. T. Coulter (Minnesota)
G. H. Wilster (Oregon)

*Subcommittee on Cheese:*

G. H. Wilster (Oregon), chairman
W. V. Price (Wisconsin)
A. J. Morris (Utah)
E. F. Goss (Iowa)

*Subcommittee on Ice Cream:*

W. H. Martin (Kansas), Chairman
W. E. Peterson (Minnesota)
L. K. Crowe (Nebraska)
P. S. Lucas (Michigan)

*Subcommittee on Condensed and Evaporated Milk:*

W. D. Swope (Pennsylvania), Chairman
E. O. Anderson (Connecticut)
L. M. Thurston (West Virginia)
E. O. Herreid (Minnesota)

*Subcommittee on Dry Milk:*

F. J. Doan (Pennsylvania), Chairman
J. L. Hileman (Dairymen's League, Syracuse, N. Y.)
G. C. Supplee (Dry Milk Co., Bainbridge, N. Y.)
R. W. Titus (Nestle's Milk Products Co., Marysville, Ohio)
J. I. Keith (Oklahoma)

*Subcommittee on Skimmilk, Buttermilk, and Whey:*

B. L. Herrington (Cornell), Chairman
B. E. Horrall (Purdue)
E. W. Bird (Iowa)
A. H. Johnson (National Dairy Products Corp., Baltimore, Md.)

*Committee on Bacteriological Methods for the Analysis of Milk and Dairy Products:*

H. Macy (Minnesota), Chairman
E. H. Parfitt (Purdue)
E. G. Hood (Ottawa, Canada)
A. C. Fay (Kansas)
P. A. Downs (Nebraska)
P. S. Prickett (Mead, Johnson & Co., Evansville, Ind.)
R. S. Breed (Geneva, N. Y.)

*Subcommittee on Butter:*

E. H. Parfitt (Purdue), Chairman
B. W. Hammer (Iowa)
E. G. Hood (Ottawa, Canada)
R. P. Myers (National Dairy Products Corp., Baltimore, Md.)

*Subcommittee on Cheese:*

E. G. Hood (Canada), Chairman
W. C. Frazier (Wisconsin)

W. S. Golding (Idaho)
W. V. Price (Wisconsin)
C. D. Kelly (Old Tavern Farms, Inc., Portland, Me.)

*Subcommittee on Ice Cream:*

A. C. Fay (Kansas), Chairman
B. W. Hammer (Iowa)
F. W. Fabian (Michigan)

*Subcommittee on Condensed and Evaporated Milk:*

P. A. Downs (Nebraska), Chairman
B. W. Hammer (Iowa)
W. A. Cordes (Blue Valley Creamery Co., Chicago)

*Subcommittee on Dry Milk:*

P. S. Prickett (Mead, Johnson & Co., Evansville, Ind.), Chairman
G. C. Supplee (Dry Milk Co., Bainbridge, N. Y.)
R. Schneiter (U. S. D. A.)

*Subcommittee on Market Milk:*

R. S. Breed (Geneva, N. Y.), Chairman
J. A. Anderson (New Jersey)
H. E. Bremer (Montpelier, Vt.)
M. W. Yale (Geneva, N. Y.)
C. K. Johns (Ottawa, Canada)
C. N. Stark (Cornell)

# AMERICAN DAIRY SCIENCE ASSOCIATION

*The Thirtieth Annual Meeting*

St. Paul, Minnesota, June 24 to 27, 1935

## GENERAL PROGRAM

*Monday, June 24*

9 A. M.–5 P. M.—General registration and room registration. Haecker Hall, University Farm.

1:30 P. M.–4:30 P. M.—Extension Section, Haecker Hall, Room 210.

2 P. M.—Dairy products judging and special butter judging demonstration by federal butter graders. Haecker Hall, Creamery Room

3 P. M.—Executive Committee Meeting, Haecker Hall, Room 214.

5 P. M.–9 P. M.—General registration and room registration. Women's Dormitory, University Farm.

7:30 P. M.—Opening Session and Business Meeting. Auditorium, Administration Building, University Farm.
President C. L. Roadhouse, presiding.
Address of Welcome—President L. D. Coffman, University of Minnesota.
Address of Welcome—Dean W. C. Coffey, University of Minnesota.
*Business Meeting.*

8:30 P. M.—Informal social get-together for members and guests and their families, Home Economics Building, University Farm.

*Tuesday, June 25*

8 A. M.–4 P. M.—General registration and room registration. Haecker Hall, University Farm.

9 A. M.–12 NOON—Joint Meeting of Section O, American Association for the Advancement of Science, American Dairy Science Association, Corn Belt Section of the American Society of Agronomy, American Society of Horticultural Science, Great Plains Section, American Phytopathological Society, The Genetics Society of America, and The American Society of Plant Physiologists. Auditorium, Administration Building, University Farm.
Symposium: Improving the Germ Plasm of Domestic Plants and Animals, H. K. Hayes, Chairman, Section O, Vice President, A. A. A. S., presiding.

Introductory Address: Dean W. C. Coffey, University of Minnesota.
Some Accomplishments with Animals: Dr. J. L. Lush, Iowa State College.
Analysis and Synthesis in the Development of New Varieties of Plants: Dr. O. S. Aamodt, University of Wisconsin.

12 NOON–1 P. M.—Lunch hour.
1 P. M.–2 P. M.—Extension Exhibits, Haecker Hall, Room 101.
1 P. M.–4 P. M.—Production Section, Haecker Hall, Room 100.
1 P. M.–4 P. M.—Manufacturing Section, Haecker Hall, Room 109.
4 P. M.–4:30 P. M.—Section Committee Meetings.
4 P. M.–4:30 P. M.—Members, guests and their families leave for trip to Land-O-Lakes Creameries, Inc., Minneapolis.
6 P. M.—Complimentary dinner and entertainment as guests of Land-O-Lakes Creameries, Inc. Admission by ticket.

*Wednesday, June 26*

9 A. M.–12 NOON—Production Section. Haecker Hall, Room 100.
9 A. M.–12 NOON—Manufacturing Section. Haecker Hall, Room 109.
12 NOON–1 P. M.—Complimentary Dairy Lunch. Haecker Hall.
1 P. M.–2 P. M.—Production Section Business Meeting. Haecker Hall, Room 100.
1 P. M.–2 P. M.—Manufacturing Section Business Meeting. Haecker Hall, Room 109.
1 P. M.–2 P. M.—Extension Section Business Meeting. Haecker Hall, Room 210.
2 P. M.–4:30 P. M.—Section on Problems and Methods of Instruction. Haecker Hall, Room 100.
2 P. M.–4:30 P. M.—Manufacturing Section. Haecker Hall, Room 109.
2 P. M.–4:30 P. M.—Extension Section. Haecker Hall, Room 210.
5 P. M.—Leave for Minneapolis Automobile Club.
7 P. M.—Subscription banquet and entertainment at Minneapolis Automobile Club. (Tickets to be purchased on registration.)

*Thursday, June 27*

9 A. M.–10 A. M.—Closing general session and business meeting. Auditorium, Administration Building, University Farm. President C. L. Roadhouse presiding.
10 A. M.–1 P. M.—Production Section. Haecker Hall, Room 100.
10 A. M.–1 P. M.—Manufacturing Section. Haecker Hall, Room 109.
1 P. M.–2 P. M.—Lunch hour.
2 P. M.—Leave for arranged tours to cooperative and independent dairy plants in the Twin Cities and trips to farms of various breeders in the vicinity of the Twin Cities. Information regarding these tours and trips will be available on registration. Members desiring any given tour or trip will register for same at time of general registration.

# SECTION PROGRAMS

## EXTENSION SECTION

Monday afternoon, June 24, 1:30–4:30 o'clock
Haecker Hall, Room 210

FLOYD JOHNSTON, *Chairman*

COW TESTING (A. B. Nystrom, Chairman) FEEDING (C. L. Blackman, Chairman), CALF CLUB (D. M. Seath, Chairman).
(Papers limited to 10 minutes)

E1—Revision of record forms. J. W. Linn, Kansas State College.
E2—Testers conferences—Official herd testing and testers organizations. W. T. Crandall, Cornell University.
E3—Farm account week through D. H. T. A. and bi-monthly testing. A. J. Cramer, University of Wisconsin.
E4—Analysis of D. H. I. A. data. G. A. Williams, Purdue University.
E5—A yearly association program. A. B. Nystrom, Bureau of Dairy Industry, U. S. D. A.
E6—Developing a pasture program. L. A. Higgins, Mississippi A. and M. College.
E7—A feeding program based on D. H. I. A. reports. J. O. Tretsven, Montana State College.
E8—County feeding programs. H. R. Searles, University of Minnesota.
E9—The five session feeding schools. C. L. Blackman, University of Ohio.
E10—Record keeping and reporting—Dairy club work. R. G. Connelly, Virginia A. and M. College.
E11—Basis for premium awards in dairy calf club work. Ivan McKellip, University of Ohio.
E12—Making dairymen out of club members. D. M. Seath, Kansas State College.

## PRODUCTION SECTION

Tuesday afternoon, June 25, 1:00–4:30 o'clock

Haecker Hall, Room 100

C. Y. CANNON, *Chairman*

MILK SECRETION, HORMONES, GENETICS, VITAMINS
(Papers limited to 15 minutes)

P1—The involution of the mammary gland. E. P. Reineke and C. W. Turner, University of Missouri.
P2—The ovary stimulating interaction of blood serum from cattle and gonadotrophic extracts. L. E. Casida, University of Wisconsin.
P3—A review of color inheritance in dairy cattle. Heman L. Ibsen and H. W. Cave, Kansas State College.

P4—The dietary requirements of goats for vitamin E (A progress report). John L. Wilson, B. H. Thomas and C. Y. Cannon, Iowa State College.

P5—A preliminary study of the vitamin D requirement of calves when fed natural milk as the source of this vitamin. C. F. Huffman and C. W. Duncan, Michigan State College.

P6—The pathology of rickets in dairy calves. H. Ernest Bechtel, E. T. Hollman and C. F. Huffman, Michigan State College.

P7—The color and carotene content of various home-grown roughage rations and the influence of these rations on the color, carotene and vitamin A potency of the butterfat. R. E. Hodgson, J. C. Knott, H. K. Murer and R. R. Graves, Bureau of Dairy Industry, U. S. D. A., and Washington State College.

P8—The vitamin A activity of butter produced by cows fed alfalfa hay and soybean hay cut in different stages of maturity. J. H. Hilton, J. W. Wilbur and S. M. Hauge, Purdue University.

P9—"Caratone" in rations for dairy calves. A. H. Kuhlman, W. D. Gallup and Earl Weaver, Oklahoma A. and M. College.

P10—Blindness in cattle of nutritional origin associated with constriction of the optic nerve. L. A. Moore, C. F. Huffman, and C. W. Duncan, Michigan State College.

4:00 P. M. Committee meetings.

## MANUFACTURING SECTION

Tuesday afternoon, June 25, 1:00–4:30 o'clock

Haecker Hall, Room 109

M. J. MACK, *Chairman*

### CHEMISTRY

(Papers limited to 10 minutes)

M1—Studies on the ash and nitrogen distribution of processed cheese as affected by the salts used and a comparison of the methods used for the determination of the pH of the cheese. Hugh L. Templeton, University of Wisconsin.

M2—Effects of some ions on the properties of ice cream mixes. J. I. Keith, C. W. Rink and Earl Weaver, Oklahoma A. and M. College.

M3—The hydrogen ion concentration and titratable acidity of butter, cream and buttermilk. O. F. Hunziker and W. A. Cordes, Blue Valley Creamery Co., Chicago.

M4—Range of hydrogen ion concentration in sour cream butter. E. H. Parfitt, Purdue University.

M5—The thiocyanogen value as a means of measuring unsaturated fatty acids in butterfat. O. J. Hill and L. S. Palmer, University of Minnesota.

M6—The effect of homogenization on some of the fat constants of milk. I. A. Gould and G. M. Trout, Michigan State College.

M7—Variations in physical properties of milk. G. P. Sanders, K. I. Matheson and I. A. Burkey, Bureau of Dairy Industry, U. S. D. A.

M8—Soft curd character induced in milk by intense sonic vibration. Leslie A. Chambers, University of Pennsylvania.

M9—Variations in the curd tension of the milk throughout the complete lactation period. M. H. Berry, University of Maryland.

M10—Effect of mastitis upon milk quality and composition. P. A. Downs, University of Nebraska.

M11—The relation of mastitis to the rennet coagulability and curd strength of milk. H. H. Sommers and Helene Matson, University of Wisconsin.

M12—Structural changes occurring in casein as shown by X-ray diffraction studies. S. L. Tuckey, H. A. Ruehe and G. L. Clark, University of Illinois.

4:00 P. M.—Committee Meetings.

## EXTENSION SECTION

Tuesday afternoon, June 25, 1:00–2:00 o'clock

Haecker Hall, Room 101

FLOYD JOHNSTON, *Chairman*

### EXHIBITS

Extension exhibition

Earl N. Schultz, Chairman, Iowa State College,
Ramer Leighton, University of Minnesota,
J. E. Crosby, Jr., University of Missouri.

The dairy extension exhibits from the states will be on display throughout the entire meeting. They will be discussed by the above committee during this hour. Those especially interested may extend this time.

## MANUFACTURING SECTION

Wednesday morning, June 26, 9:00–12:00 o'clock

Haecker Hall, Room 109

M. J. MACK, *Chairman*

### CHEMISTRY AND TECHNOLOGY

(Papers limited to 10 minutes)

M13—Effects of time and temperature of holding milk heat-treated at various temperatures on its subsequent coagulation by rennet. Milton E. Powell, University of Minnesota.

M14—Determinations of copper in sugar condensed milk and some relations between the copper content and off flavor in strawberry ice cream.

Harold L. Link, Harry J. Konen and L. A. Baumann, Xavier University and French-Bauer, Inc., Cincinnati.

M15—The application of the Minnesota Babcock method to the testing of ice cream, concentrated milk and chocolate milk. L. M. Thurston and W. Carson Brown, West Virginia University.

M16—The standardization of the Borden Body Flow-Meter for determining the apparent viscosity of cream. J. C. Hening, New York Agricultural Experiment Station, Geneva.

M17—Judging sweet cream. J. H. Nair, D. E. Mook and R. S. Fleming, Borden's Research Laboratory, Syracuse.

M18—Some factors affecting the properties of whipped cream. W. S. Mueller, M. J. Mack and H. G. Lindquist, Massachusetts State College.

M19—Frequency of the flavor defects in milk. Earl Weaver, E. L. Fouts and P. C. McGilliard, Oklahoma A. and M. College.

M20—Effects of feeds on oxidized flavors in pasteurized milk. Ed. Prewitt and E. H. Parfitt, Purdue University.

M21—Methods of studying feed effects on the physical properties of butterfat. Willis D. Gallup, J. I. Keith and A. H. Kuhlman, Oklahoma A. and M. College.

M22—Effect of a heavy cottonseed meal ration on milk and butter. J. I. Keith, A. H. Kuhlman, Earl Weaver and Willis D. Gallup, Oklahoma A. and M. College.

## PRODUCTION SECTION

Wednesday morning, June 26, 9:00–12:00 o'clock

Haecker Hall, Room 100

C. Y. CANNON, *Chairman*

VITAMINS (continued), SILAGE, HAY
(Papers limited to 15 minutes)

P11—A study of the malnutrition incident to the heavy feeding of cottonseed meal. S. I. Bechdel and S. R. Skaggs, Pennsylvania State College.

P12—Influence of the ration on the vitamin C content of milk. W. H. Ridell, C. H. Whitnah and J. S. Hughes, Kansas State College.

P13—Effect of the condition of corn plant at cutting upon the carotene content of silage. E. A. Kane and C. A. Cary, Bureau of Dairy Industry, U. S. D. A.

P14—The stack silage method of preserving forage crops and the comparative nutritive value of oat and pea silage made in a stack and in a tower. J. C. Knott, R. E. Hodgson and R. R. Graves, Washington State College and Bureau of Dairy Industry, U. S. D. A.

P15—Pea vine silage as a feed for dairy cattle. J. C. Tretsven, Montana State College.

P16—Studies regarding the use of mineral acids for the preservation of forage. C. C. Hayden, A. E. Perkins, C. F. Monroe, W. E. Krauss,

C. E. Knoop, R. G. Washburn and T. S. Sutton, Ohio Agricultural Experiment Station, Wooster.

P17—Molasses as a preserving agent in making soybean silage. E. C. Elting, Clemson College, South Carolina.

P18—Experimental cottonseed meal ration plus silage versus herd ration, hay and silage feeding. R. H. Lush, Louisiana State University.

P19—The digestibility and feeding value of Russian thistle hay. H. W. Cave, W. H. Riddell and J. S. Hughes, Kansas State College.

P20—*Lespedeza Sericea* feeding trials with dairy cows. C. E. Wylie and S. A. Hinton, University of Tennessee.

## MANUFACTURING SECTION

Wednesday afternoon, June 26, 1:00–4:30 o'clock

Haecker Hall, Room 109

C. J. MACK, *Chairman*

### BACTERIOLOGY

(Papers limited to 10 minutes)

1 P. M.–2 P. M.—Section business meeting.

M23—The detection and control of bovine mastitis. G. J. Hucker, New York Agricultural Experiment Station, Geneva.

M24—Studies on aseptically drawn milk from Bang's disease positive and Bang's disease negative cows. H. B. Morrison and F. E. Hull, University of Kentucky.

M25—Bitter flavor in cheddar cheese from pasteurized milk. C. A. Phillips, University of California.

M26—Study of a gassy defect in cream cheese. W. J. Corbett, W. C. Frazier and W. V. Price, University of Wisconsin.

M27—Varieties of the genus Oospora found in cream and butter. C. M. Sorensen, Purdue University.

M28—The disappearance of acetylmethylcarbinol and diacetyl in butter cultures. G. L. Stahly, M. B. Michaelian, C. H. Werkman and B. W. Hammer, Iowa State College.

M29—A study of *Escherichia-aerobacter* organisms in pasteurized milk. Joseph L. Minkin and L. H. Burgwald, Ohio State University.

M30—Observations on yeasts causing gas in sweetened condensed milk. H. C. Olson and B. W. Hammer, Iowa State College.

M31—Standard laboratory methods for the control of dairy products. Robert S. Breed, New York Agricultural Experiment Station, Geneva.

## EXTENSION SECTION

Wednesday afternoon, June 26, 1:00–4:30 o'clock

Haecker Hall, Room 210

FLOYD JOHNSTON, *Chairman*

SIRES (J. G. KENDRICK, *Chairman*) QUALITY IMPROVEMENT
(A. C. BOLTZER, *Chairman*)
(Papers limited to 10 minutes)

1 P. M.–2 P. M.—Section business meeting.

E13—Listing D. H. I. A. proved sires—Permanent records. E. N. Schultz, Iowa State College.

E14—Terminology for indicating the merits of D. H. I. A. sires. S. J. Brownell, Cornell University.

E15—Analysis of D. H. I. A. proved sire records. E. J. Perry, State University of New Jersey.

E16—Sons of D. H. I. A. proved sires. D. H. Fourt, University of Idaho.

E17—Standardized lactation records for reporting dam and daughter comparisons in D. H. I. A. J. F. Kendrick, Bureau of Dairy Industry, U. S. D. A.

E18—Quality improvement committee report. E. C. Scheidenhelm, University of Nebraska.

E19—Quality improvement committee report. C. A. Hutton, University of Tennessee.

E20—Quality improvement committee report. Fred H. Abbott, University of California.

E21—Quality improvement committee report. A. J. Mann, Connecticut Agricultural College.

E22—Quality improvement committee report. A. C. Baltzer, Michigan State College.

## SECTION ON PROBLEMS AND METHODS OF INSTRUCTION

Wednesday afternoon, June 26, 2:00–4:30 o'clock

Haecker Hall, Room 100

EARL WEAVER, *Chairman*
(Papers limited to 15 minutes)

I1—A basic curriculum for an agricultural college. H. P. Davis, University of Nebraska.

I2—Certain suggestions for a course of study for those majoring in dairy industry. J. H. Frandsen, Massachusetts State College.

I3—Building a course in dairy husbandry. H. P. Davis, University of Nebraska.

I4—Frequent quizzes in teaching dairy elements. E. L. Fouts and J. I. Keith, Oklahoma A. and M. College.

I5—Adjusting dairy instruction to the needs of the state. C. E. Wylie, University of Tennessee.

I6—The integrated course of study in agriculture. A. M. Field (by invitation) Division of Agricultural Education, University of Minnesota.

## PRODUCTION SECTION

Thursday morning, June 27, 10:00–1:00 o'clock

Haecker Hall, Room 100

C. Y. CANNON, *Chairman*

### NUTRITION, RATIONS, PERFORMANCE

(Papers limited to 15 minutes)

P21—The effect of soybeans on the fat content of milk. J. W. Wilbur, J. H. Hilton and co-workers, Purdue University.

P22—The effect of quality and level of protein intake on growth and milk production. I. W. Rupel, G. Bohstedt and E. B. Hart, University of Wisconsin.

P23—Milk and butterfat production on high and low protein rations. C. A. Cary, Bureau of Dairy Industry, U. S. D. A.

P24—Extremes in protein feeding—Bearing of results on the protein-feeding standard. A. E. Perkins, Ohio Agricultural Experiment Station.

P25—Changes in weight of new born calves as related to the method of feeding. C. L. Cole, University of Minnesota.

P26—The nutrients required by dairy cows kept in an open shed versus cows kept in a dairy barn. J. R. Dice, North Dakota Agricultural College.

P27—Formulae for calculating rations for milk cows. A. H. Kuhlman, Oklahoma A. and M. College.

P28—Permanent records in the station herd. R. B. Becker, University of Florida.

P29—Effect of breeding efficiency and culling on herd production. Lynn Copeland, American Jersey Cattle Club, New York City.

## MANUFACTURING SECTION

Thursday morning, June 27, 10:00–1:00 o'clock

Haecker Hall, Room 109

C. J. MACK, *Chairman*

### CHEESE, BUTTER AND TECHNOLOGY

(Papers limited to 10 minutes)

M32—Acidity in the manufacture of cream cheese. Z. D. Roundy and W. V. Price, University of Wisconsin.

M33—The manufacture of a soft cheese of the Bel Paese type. Robert R. Farrar, Bureau of Dairy Industry, U. S. D. A.

M34—Experiments with canned cheddar cheese. E. L. Reichart, University of Nebraska.

M35—The vitamin A content of sour cream butter, sweet cream butter and margarine. I. L. Hathaway and H. P. Davis, University of Nebraska.

M36—Technic, examination and reporting extraneous matter in butter. B. E. Horrall, Purdue University.

M37—Notes on the national cream quality improvement campaign. M. E. Parker, Seal-test System Laboratories, Inc., Cleveland.

M38—Efficiency of electrically operated tanks versus ice in the cooling of milk. J. H. Frandsen, Massachusetts State College.

M39—Frozen brines as refrigerants of ice cream in cabinets and shipping containers. H. H. Sommer, University of Wisconsin.

M40—An experimental ice cream freezing unit. J. I. Keith and C. W. Rink, Oklahoma A. and M. College.

M41—Methods for testing condensing pans. L. C. Thomsen, University of Wisconsin.

# AMERICAN DAIRY SCIENCE ASSOCIATION

INCORPORATED IN THE DISTRICT OF COLUMBIA

*Officers*

*President* ..... C. L. ROADHOUSE, Davis, Calif.
*Vice-President* ..... H. A. RUEHE, Urbana, Ill.
*Secretary-Treasurer* ..... R. R. GRAVES, Washington, D. C.
*Editor* ..... A. C. DAHLBERG, Geneva, N. Y.
*Director* ..... M. MORTENSEN, Ames, Iowa.
*Director* ..... O. F. HUNZIKER, Chicago, Illinois
*Director* ..... L. A. ROGERS, Washington, D. C.

*Officers of Sections*

SECTION NO. 1—Dairy Production

*Chairman* ..... C. Y. CANNON, Ames, Iowa
*Secretary* ..... R. H. LUSH, Baton Rouge, La.

SECTION NO. 2—Dairy Manufactures

*Chairman* ..... M. J. MACK, Amherst, Mass.
*Secretary* ..... G. M. TROUT, East Lansing, Mich.

SECTION NO. 3—Extension

*Chairman* ..... FLOYD JOHNSON, Ames, Iowa.
*Secretary* ..... C. L. BLACKMAN, Columbus, Ohio

*Officers of Divisions*

*Southern* ..... R. H. LUSH, Chairman, Baton Rouge, Louisiana
A. H. KUHLMAN, Secretary, Stillwater, Okla.

*Eastern* ..... R. E. JOHNSON, Chairman, Storrs, Conn.
H. G. LINDQUIST, Amherst, Mass.

*Western* ..... J. A. NELSON, Chairman, Bozeman, Mont.
O. J. HILL, Secretary, Pullman, Wash.

---

*The American Dairy Science Association* was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership shall consist of two kinds: (1) active, (2) associate.

The qualifications for membership in the two classes are as follows: (a) Any person is eligible to active membership who is formally announced by an Agricultural College, or Experiment Station, or by the Bureau of Dairying of the United States Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or (b) anyone filling a position of responsibility connected with the dairy industry and who has had a college or University training in technical science, or anyone filling a responsible position in the industry of a professional character requiring a technical knowledge of dairying of a high order.

Any person is eligible to associate membership who is regularly enrolled in a collegiate course in a college of Agriculture and who is specializing in dairying. Associate membership is attained by election to membership in a local chapter of the American Dairy Science Association.

The dues are $5.00 a year for active membership. Correspondence regarding membership and dues should be addressed to R. R. Graves, United States Department of Agriculture, Washington, D. C.

# B-K

## Helps Dairymen Produce *High Quality* MILK

B-K is needed now, more than at any other time of the year, to help prevent off-flavor, quick-souring and high bacteria count milk.

By thoroughly cleaning utensils after milking . . . by properly cooling milk . . . by sterilizing utensils *just before using* with B-K, high quality milk can be produced in spite of hot weather!

B-K, the preferred sterilizer with thousands of dairy farmers and milk plant managers, *destroys instantly on contact* 99% of all bacteria on milking utensils and equipment that cause odors, off-flavor and quick souring of milk.

### *B-K Comes in Both Powder and Liquid Form*

B-K Powder contains 50% available chlorine and can be used either to make a stock solution or added direct to rinse water. B-K Liquid is still preferred by thousands of users because it provides a dependable, ready-made solution at a low price.

*Send today for quantity discounts and valuable free book, "Better Dairy Products."*

**General Laboratories, Inc.**

Dept. 62, Widener Building, Philadelphia, Pa.

**IMPORTANT**

**B-K sterilizes efficiently in cool, warm or hot water. Tests proved that solutions of B-K containing 50, 100 and 200 p.p.m. when boiled for 10 minutes, did not lose their available chlorine content. Laboratory data on heat tests free on request.**

Chr. Hansen's Laboratory in Milwaukee

## USING THE MOST MODERN METHODS

It is of utmost importance to the dairy industry that it have preparations of dependable quality and known uniformity. To provide such assurance, we operate this completely modern laboratory where Hansen's Rennet, Cheese Color, Butter Color and Lactic Ferment Cultures are prepared under constant scientific control.

**CHR. HANSEN'S LABORATORY, INC.**

Milwaukee, Wis.

## THE SCIENCE PRESS PRINTING COMPANY

Printers of Scientific and Educational Journals, Monographs and Books

Information furnished and detailed estimates submitted

Lime and Green Streets
Lancaster, Pennsylvania

**Your advertisement is being read in every State and in 25 Foreign Countries**

# TO TEST BUTTER AND CREAM *for* EXTRANEOUS MATTER

WE recommend two Spencer Microscope equipments. Equipment "A" is suitable for routine work but is limited to one magnification, 81X, which is not enough to identify cat hair. Equipment "B," preferred by most users, has variable magnifications of 81X, 41X and 16X. Rat hair can be easily identified.

The specific advantages of the Spencer No. 60 for Microscopy as used to examine the filter pad are:

(1) The image is NOT inverted nor reversed.
(2) The image is stereoscopic and third dimensional.
(3) Free from eyestrain.
(4) Wide Field.

*Write us for folder M 67 for complete information and description. Address Dept. W-2.*

Spencer Lens Company

BUFFALO NEW YORK



# JOURNAL OF DAIRY SCIENCE

## Official Organ of American Dairy Science Association

*Agents in Foreign Countries*

*For Argentina and Uruguay:* Bestelspanner y Cia, Sarmiento 615, Buenos Aires.

*For Australia:* Angus & Robertson, Limited, 89-97 Castlereagh Street, Sydney; Stirling & Co., 317 Collins Street, Melbourne.

*For Belgium:* Henri Lamertin, 53 Rue Candenberg, Bruxelles.

*For the British Empire, except Australia and Canada:* Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, W. C. 2, London.

*For Canada:* Wm. Dawson & Son, Ltd., 67 Queen Street East, Toronto.

*For China:* Commercial Press Ltd., Pawhan Road, Shanghai, China.

*For Denmark:* H Hagerup's Bogtrykkeri, Gothersgade 30, Kjöbenhavn.

*For France:* Paulin Dougnac, 47 Rue des Ecoles, Paris.

*For Germany:* G. Westermann Co., Inc., Kronenstrasse 15, Berlin NW 52, Germany.

*For Holland:* Scheltema & Holkema, Boek 74-76, Amsterdam.

*For Japan and Korea:* Maruzen Company, Ltd. (Maruzen Kabushiki Kaisha), 12 to 16 Nihonbashi Tori-San-Chome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

*For Spain:* Bailly Hermanos, Plaza de Santa Ana, 25, Madrid.

Subscription price is $5.00 per volume, postpaid, except in Canada and countries within the Postal Union where the price is $5.06.

The Science Press Printing Company,
Lancaster, Pa., U. S. A.

WYANDOTTE AA31
ALUMINUM CLEANER 4X
WYANDOTTE DETERGENT *do the* *Unusual*

# CLEANING JOBS

Several members of the family of Wyandotte Products are especially made to do the unusual cleaning jobs around the dairy.

For brightening equipment and removing baked-on milk substances (commonly called milk stone) use *Wyandotte AA31*. It is readily and completely soluble, economical to use, and meets any hard water conditions.

*Aluminum Cleaner 4X* will not tarnish or corrode aluminum and can be used in hard water. It rinses freely.

Try *Wyandotte Detergent* for polishing equipment and cans. Perhaps you can save re-tinning expense.

WRITE FOR FULL INFORMATION

THE J. B. FORD COMPANY Wyandotte, Michigan

Difco

## Ingredients for the Standard Plating Medium

The medium recommended in "Standard Methods of Milk Analysis" for the routine plate count of milk may be prepared from Bacto-Beef Extract, Bacto-Peptone and Bacto-Agar without adjustment of the reaction or filtration of the medium. These ingredients are prepared especially for use in bacteriological culture media.

### Bacto-Beef Extract

Recommended in "Standard Methods." Forms unobtrusively clear solutions in the concentrations usually employed. Has a reaction of pH 6.6.

### Bacto-Peptone

Solutions are sparklingly clear and neutral in reaction. Recommended in "Standard Methods."

### Bacto-Agar

Free from extraneous matter, yielding clear media which require no filtration. In convenient, granular form.

Specify "Difco"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN